



User's Manual
[Quick Start]

FLUOVIEW
FV1000
LASER SCANNING
BIOLOGICAL MICROSCOPE

FV10-ASW [Ver2.0]

Notice

Thank you for your purchase of Olympus microscope at this time.
Hold this manual by your side when using this microscope all the time and keep it with care after reading.

AX7797

Caution

FV1000MPE is a CLASS 4 laser product; FV1000 is a CLASS 3B laser product.

The procedures for using this system are classified as follows:

- Service

“Service” means any adjustment or repair performed by highly trained and skilled technical personnels who are provided the service training following to the service manual for this system.

The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B or CLASS 4 laser light is emitted.

- Maintenance

“Maintenance” means adjustment or other procedures performed by customers to maintain that this system functions properly.

- Operation

“Operation” means all performance described in the user’s manuals in this system.

CLASS 3B or CLASS 4 laser light is only emitted from the objective lens during the actual execution.

The User’s Manuals of this system consist of the following:

In order to maintain the full performance of this system and ensure your safety, be sure to read these user’s manuals and the operating instructions for the laser unit and light source unit before use.

User’s manual constitution of FV1000MPE

- FV1000MPE / FV1000 User’s Manual [Laser Safety Guide]
- FV1000MPE User’s Manual [Safety Manual] or [Safety Guide]
- FV1000 User’s Manual [Safety Guide]
- FV1000MPE User’s Manual [Operation Manual] or [Operation]
- FV1000 User’s Manual [Hardware Manual]
- FV1000 FV10-ASW User’s Manual [Quick Start]

User’s manual constitution of FV1000

- FV1000MPE / FV1000 User’s Manual [Laser Safety Guide]
- FV1000 User’s Manual [Safety Guide]
- FV1000 User’s Manual [Hardware Manual]
- FV1000 FV10-ASW User’s Manual [Quick Start]

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service require to take the service training.

- FV1000MPE / FV1000 Service Manual



In case of purchasing the laser simultaneously, we have prepared the following manual for the laser.

- MaiTai Series User's Manual [Quick Start]

In addition, we have prepared one service manual for the laser as below. Technical personnels who perform the laser service require to take the service training.

- MaiTai Series Service Manual

Part or whole of this software as well as manual shall not be used or duplicated without consent.

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Other brand names and product names are trademarks or registered trademarks of their respective owners.

This Quick Start has divided into the volume by the following system configurations.

IX81 (Spectral Type)

IX81 (Filter Type)

BX61 (Spectral Type)

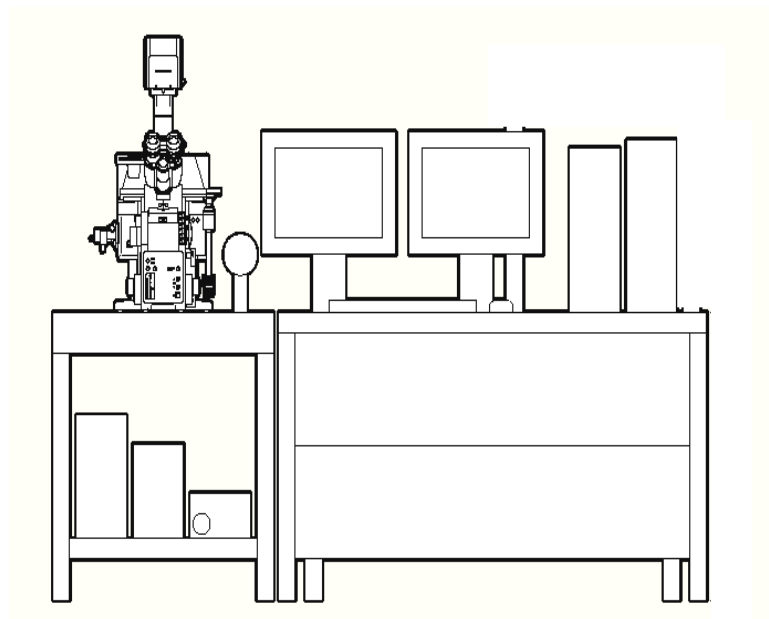
BX61 (Filter Type)



Laser Confocal Scanning Microscope

FV1000D Spectral Type
(inverted Microscope IX81)

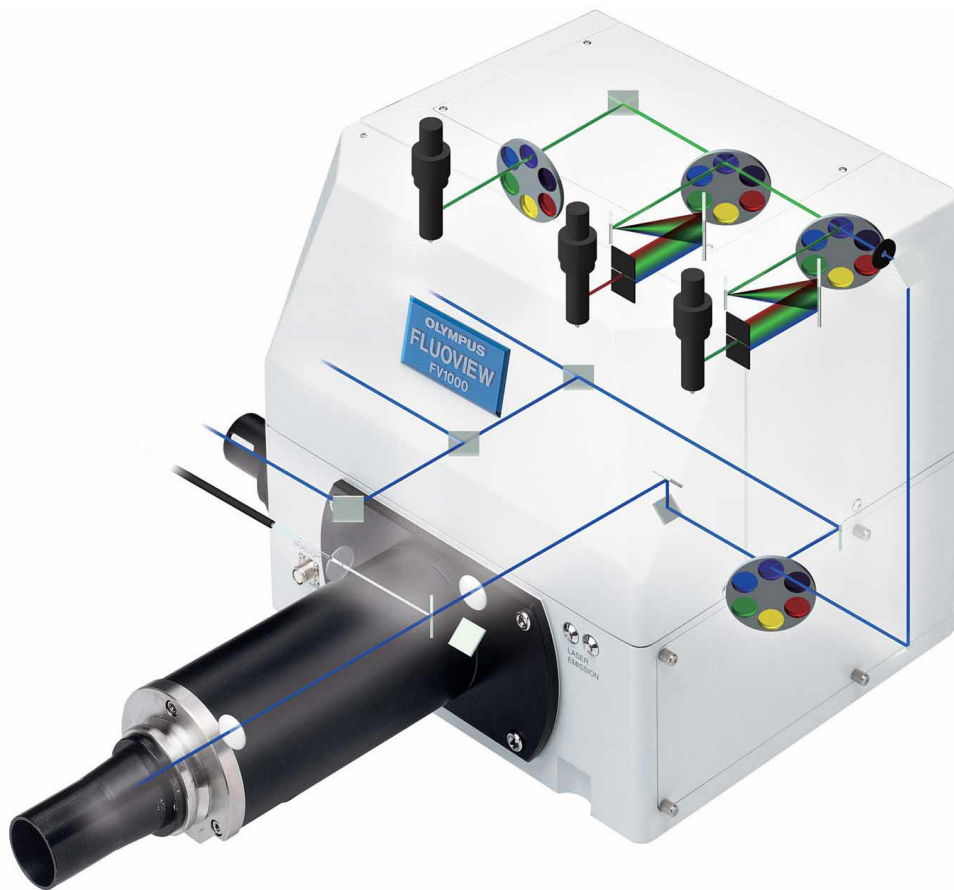
Operation Manual



Contents

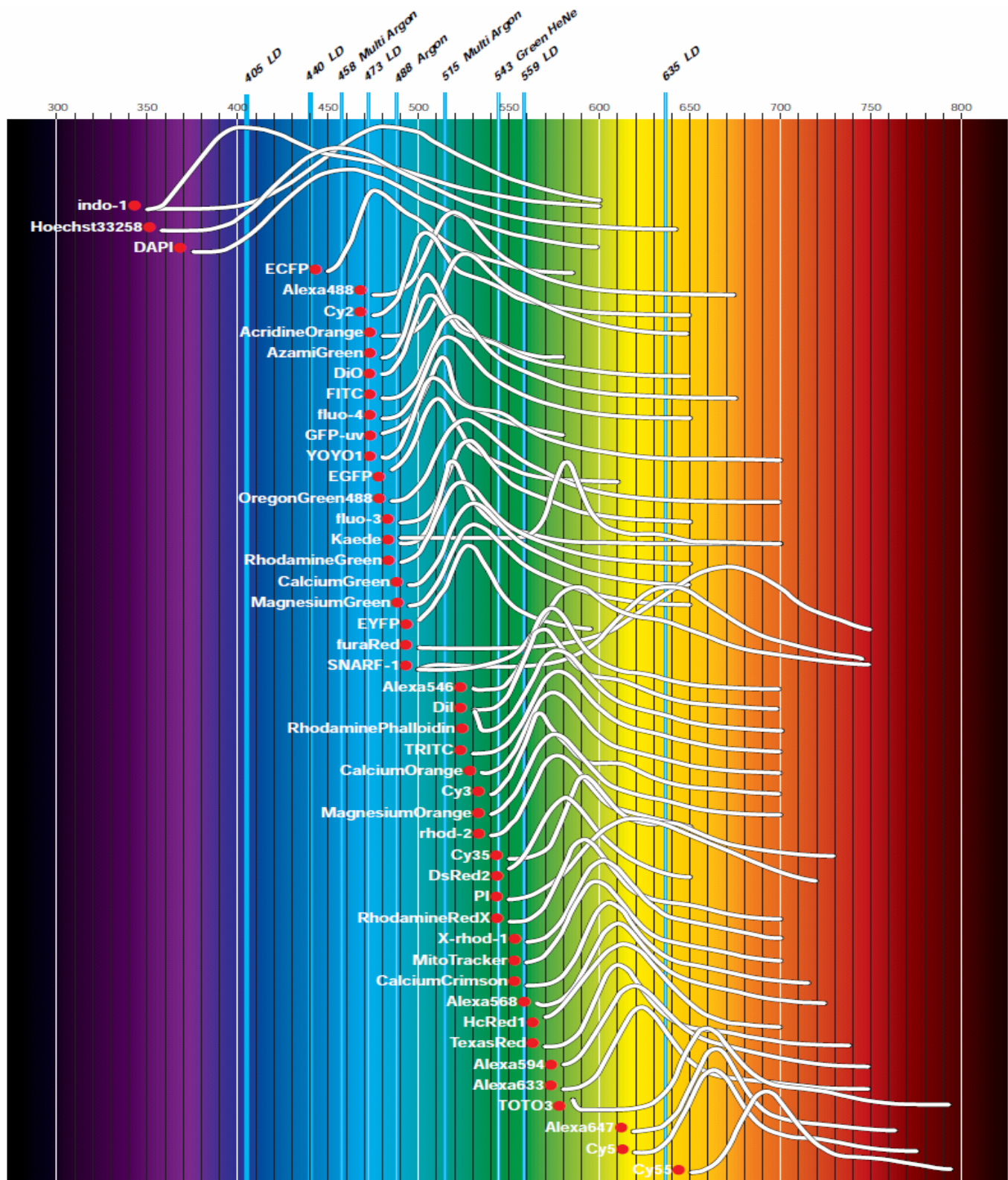
System introduction	3
FV1000D Laser DyeList	4
System Preparation	5
Visible Observation	
Observation of Fluorescence image	6
Observation of Differential Interference Contrast Images	7
Image Acquisition	
Overview of Operation Panel for Image Acquisition	8
Single Stain on XY Image	9-11
Complement of adjusting the image	12-13
Double Stain on XY Image	14-15
Sequential scan Line Sequential	16
Double Stain on XYZ image	17-18
Four Stain on XY Image	19-21
Single Stain + DIC on XY Image	22-23
Merge the image between fluorescent XY image and DIC image	24
Single Stain on XYZT Image / ZDC XYZT image	25-26
Spectral Image on XYZ Image	27-29
Unmixing	30-33
Reload the image conditions	34
Image Analysis	
Overview of the 2D Operation Panel / Opening a file	35
Making 2D Z projection file Images	36
Saving a Z section image as 2D image	37
Inserting the Scale Bar	38
Rotating a Three-dimensional Image	39
Saving an Image	40
Saving Rotating a Three-dimensional animation file	41
2D Image Analysis	
Edit the image color and contrast	42
The image of Z section	43
Intensity Profile of each Z sections	44
Measure	45
Line Intensity Profile on the 2D image / Histogram	46
Line Series Analysis / Co-localization	47
TimeLapse Analysis	48
Closing the System	48

Spectral Type Main Scanner

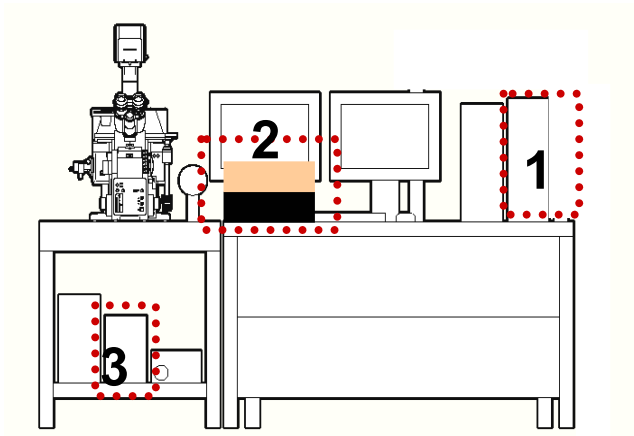


Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe(G)543nm



System Preparation



Wait for a moment until the software is started

1. Turn the computer ON.
[In case of equipped concentrated power supply, power on it first]

2. Turn the laser ON
(Turning the key switch)
2-1. LD559nm ON
2-2. Multi Ar 458nm 488nm 515nm
2-2. HeNe(G)(643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

4. Log on Windows

Enter Password, Customer name is below

User name: Administrator

Password : fluoview

5. Double click this icon
to log on to ASW

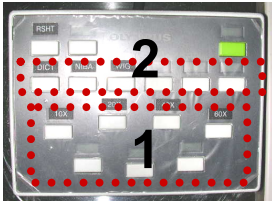


User name: Administrator

Password : Administrator

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch




1. Select an objective lens by using the hand switch
2. Select florescent filter cube

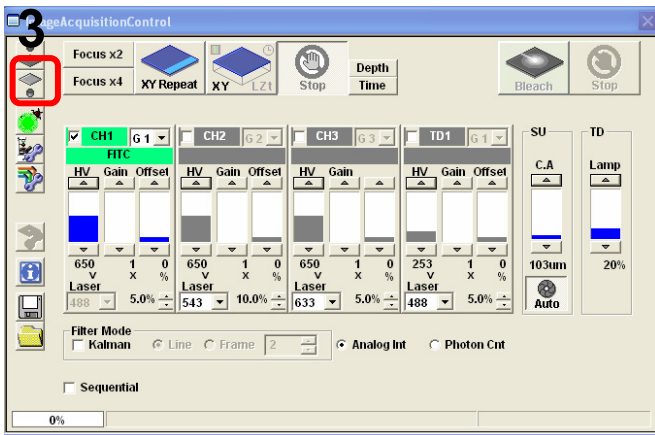
MEMO

Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)

WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.  Click the button on the Fluoview software



4. Focus to the specimen

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■



Hand switch

*DIC prism



3

Use this knob to adjust the differential interference contrast.

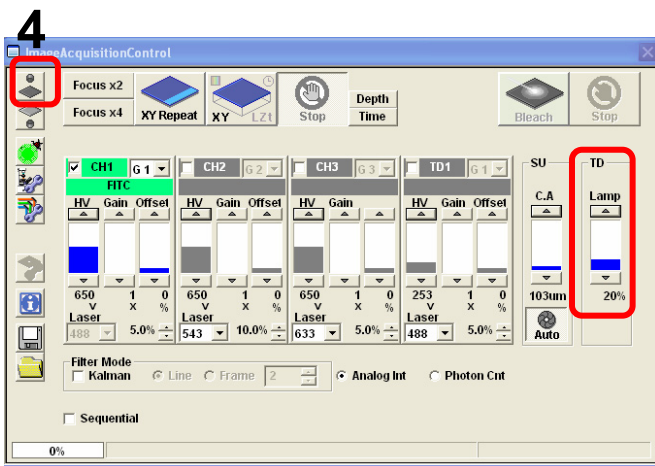
1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software

5. Focus to the specimen



Overview of Operation Panel for Image Acquisition

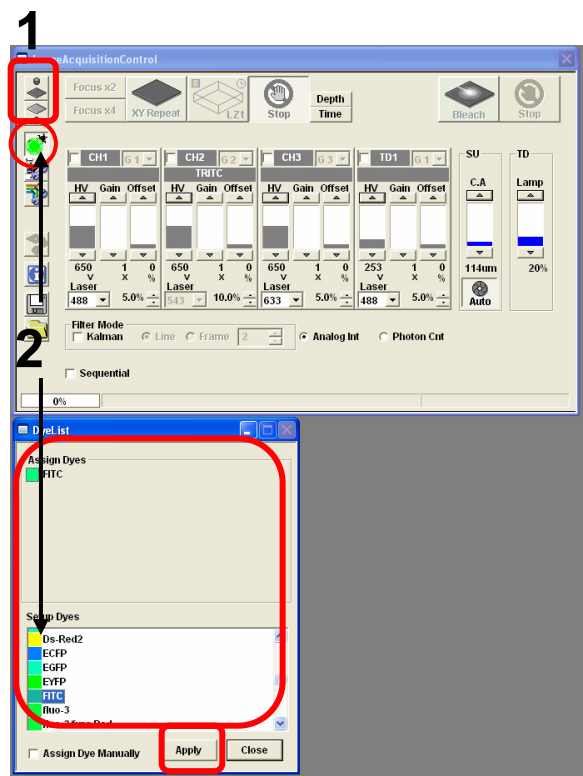
The image displays three panels of the image acquisition software interface, with various components labeled by arrows:



- AcquisitionSetting Panel:**
 - Scan mode (Mode)
 - Scan speed (Speed)
 - Number of pixels (Size)
 - Zoom & Pan (Area, PanX, PanY, Zoom)
 - Laser output adjustment (Laser)
 - Objective lens (Objective lens)
 - Focus (Focus)
 - Time Interval & Time Number (for acquisition of XYT or XT image) (TimeScan)
 - Transmitted light observation (visual observation) (Microscope)
 - Fluorescence observation (visual observation) (Microscope)
- ImageAcquisitionControl Panel:**
 - Scan buttons (Focus x2, Focus x4, XY Repeat, XY, L, Z, Stop, Lambda, Depth, Time, Bleach, Stop)
 - Select XYZ, XYT or XYL (XY, XYT, XYL)
 - Adjustment of each channel (CHS1, CHS2, CHS3, TD1)
 - Confocal aperture (C.A.)
 - Light intensity adjustment for halogen bulb (Lamp)
 - Kalman (Filter Mode)
 - Save acquisition conditions (Save)
 - Load acquisition conditions (Load)
- Image View Panel:**
 - Image display window (Image View)
 - Image file thumbnail (Image file thumbnail)
 - Display of files in the memory (Image file thumbnail)

Image Acquisition (Single Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Single stain of green fluorescence dye (FITC)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

(The DyeList panel can be closed by using the Close button.)

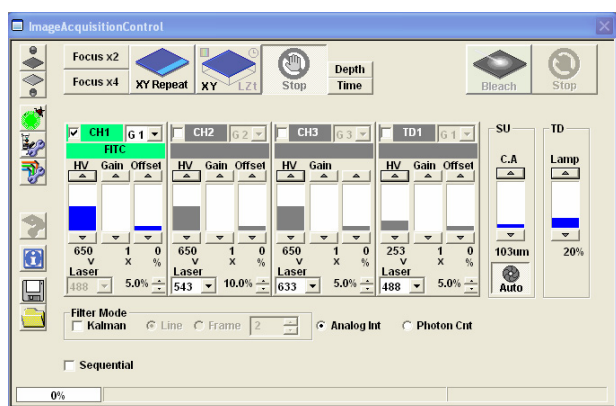
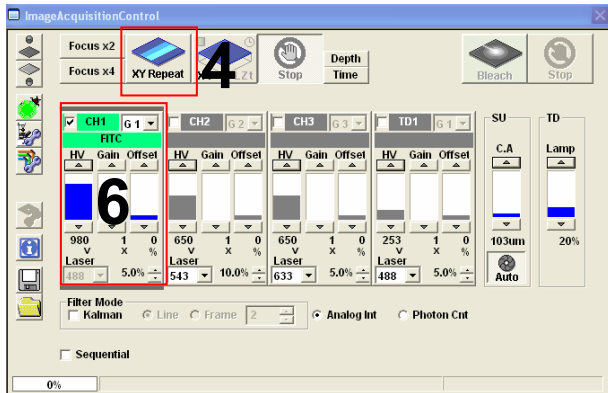


Image Acquisition (Single Stain on XY Image)



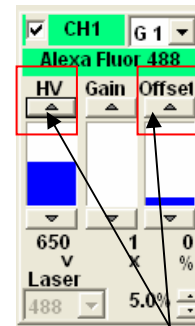
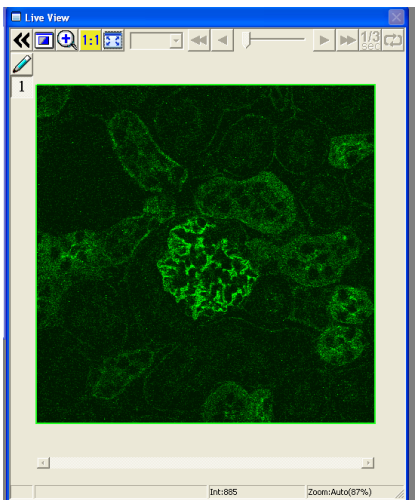
4. Press XY Repeat button click to get image



: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



Adjust sensitivity of **HV** and reduce noise by **offset**

7. Press keyboard **Ctrl + H key**

Optimized PMT adjustment brightness intensity 2 color between white and black,

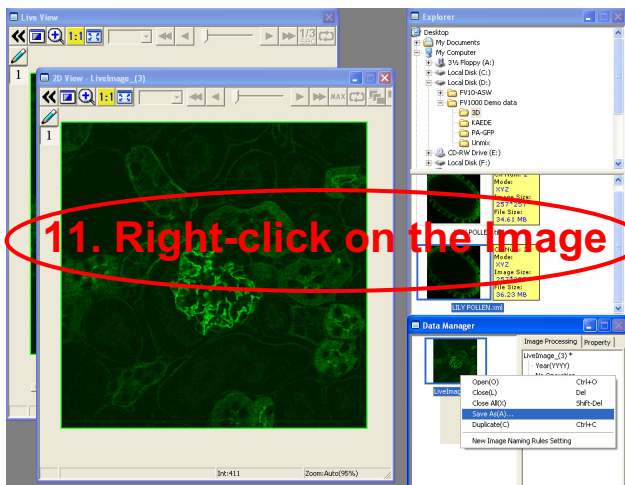
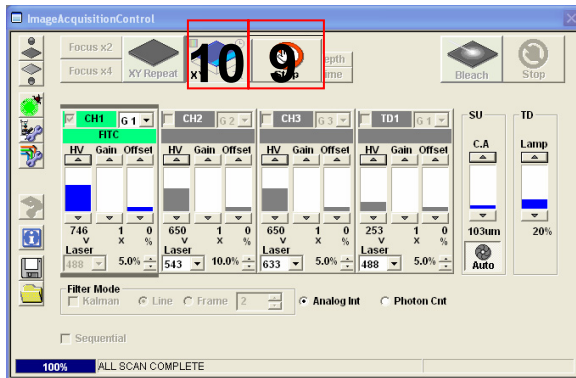
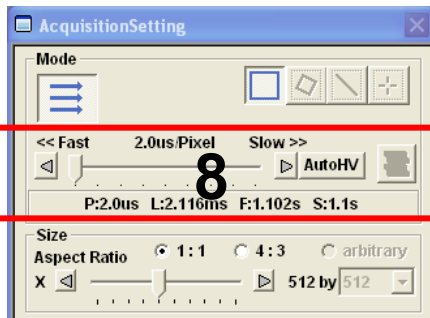
Maximum intensity is 4095(12bit) if intensity is over 4095, color is changed to red (saturation)

* Basically, **Gain value is 1**





7

Image Acquisition (Single Stain on XY Image)



8. Select AutoHV and then select ScanSpeed.
*As the scan speed becomes slower, noise can be removed while maintaining the current brightness.

9.  Press the Stop button to stop scanning.

10.  Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

11. Saving the image:

Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

Save the image as TIFF,BMP,JPEG format Select "Export" and chose the format TIFF, BMP, JPEG.

■Memo■

File formats specifically for the FV10-ASW

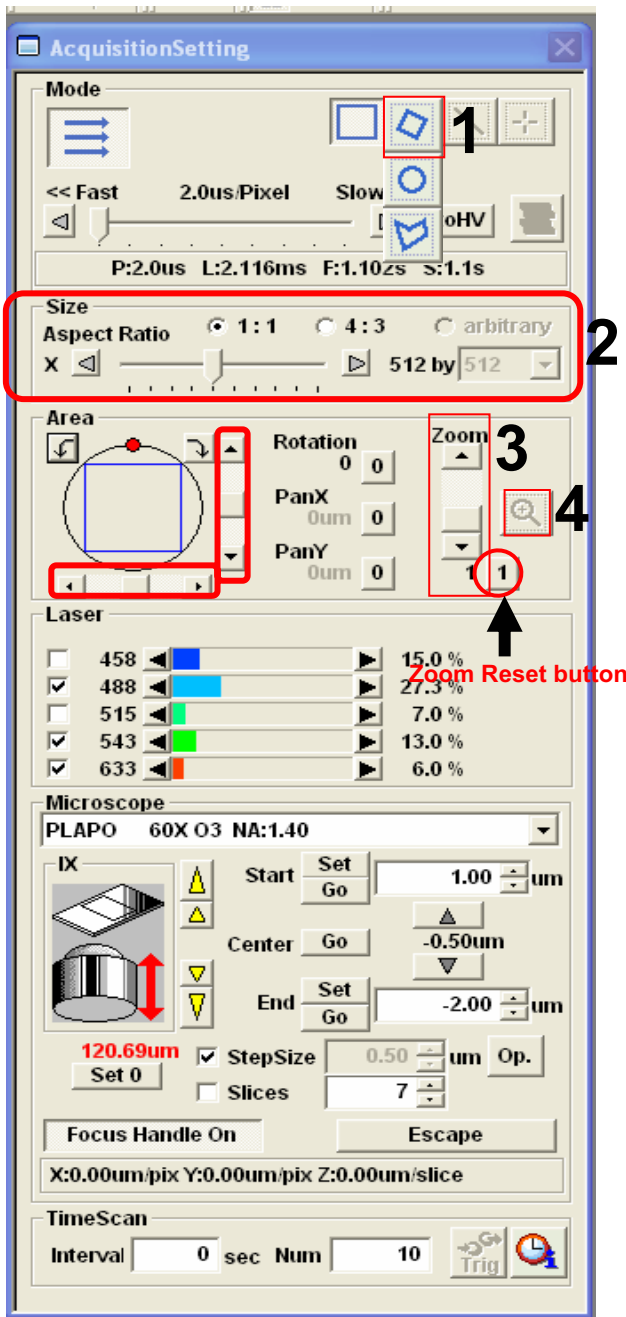
OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

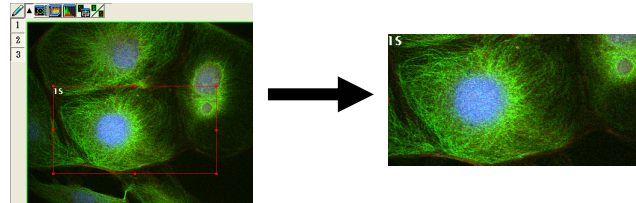
OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Complement of adjusting the image



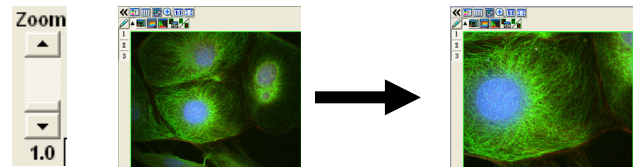
1. Click "Clip scan" button , and enclose an interesting region's image on the whole image.




2. pixel setting
* The standard pixel is 512 x 512

3. Zoom Setting

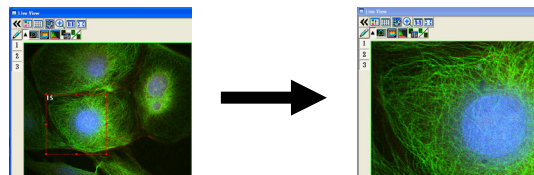
Press "XY Repeat" to scan and set zoom value.



Above image is zoomed From 1 to 2
* Scan speed and pixel resolution remain even zoom value is changed

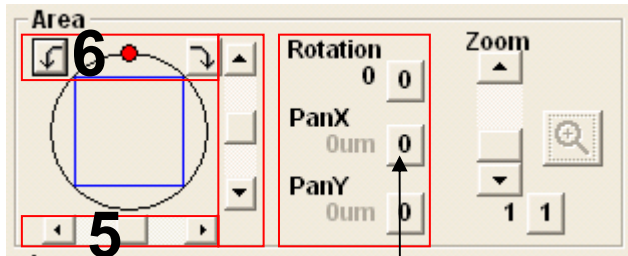
4. Click  Zoom scan, and be able to enclose an interesting region's on the whole image

Press XYRepeat to scan after enclose the region

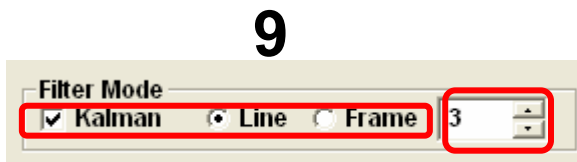
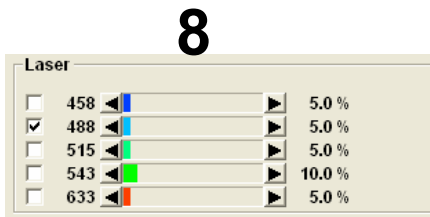


* Scan speed and pixel resolution remain even zoom value is changed

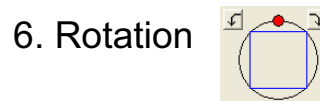
Complement of adjusting the image




PanX,Y and Rotation reset button



Be able to move the field of view to set Pan X,Y without stage action



Be able to rotate the whole image.

7.  Click "Auto" button to acquire Optimized Conforcal aperture Conforcal aperture . . . change conforcal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity . . . More Laser intensity is increase , more bright image is .

* More increase laser intensity is , more discoloration image is .

9. Kalman accumulation . . . Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

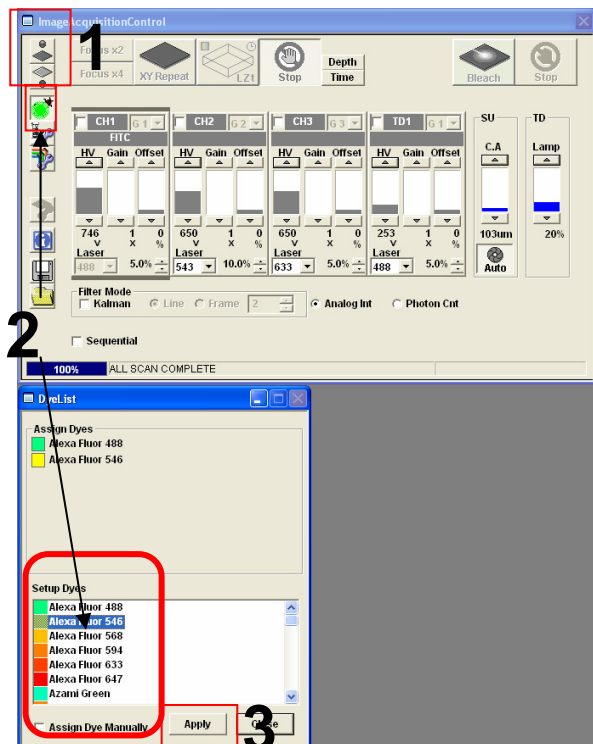
Disadvantage: Some blur occurs due to averaging of images.



Image Acquisition (Double Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



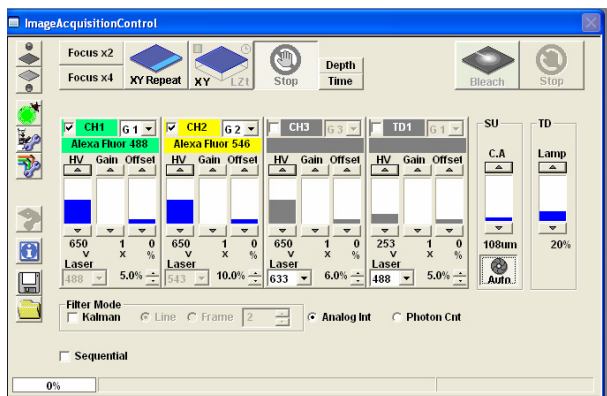
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

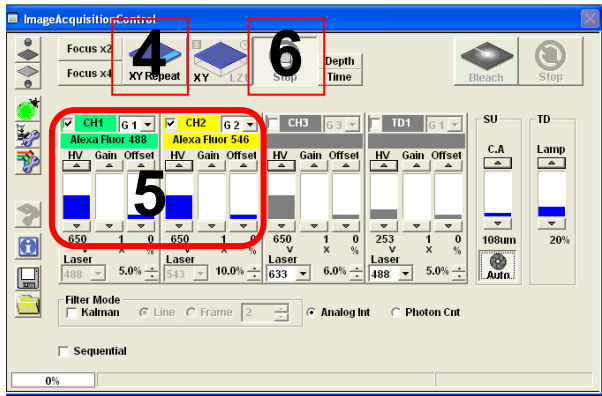
3. Click “Apply” button.

(The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

Image Acquisition (Double Stain on XY Image)



4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

(The image adjustment is outlined below.
For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■

Scan buttons



: Continuous scan

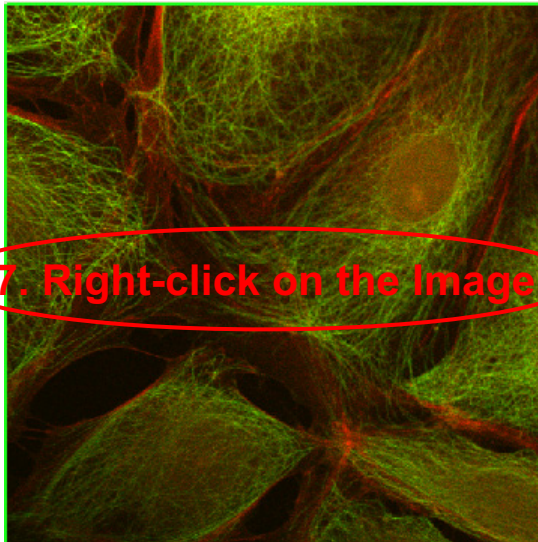


: Stop scan



: Rough scan (Line skipped)

7. Right-click on the Image



7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

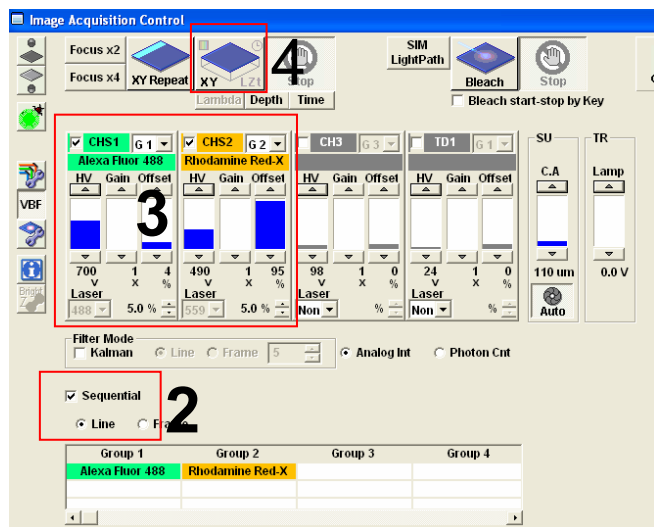
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

Image Acquisition (Double Stain on XY Image)

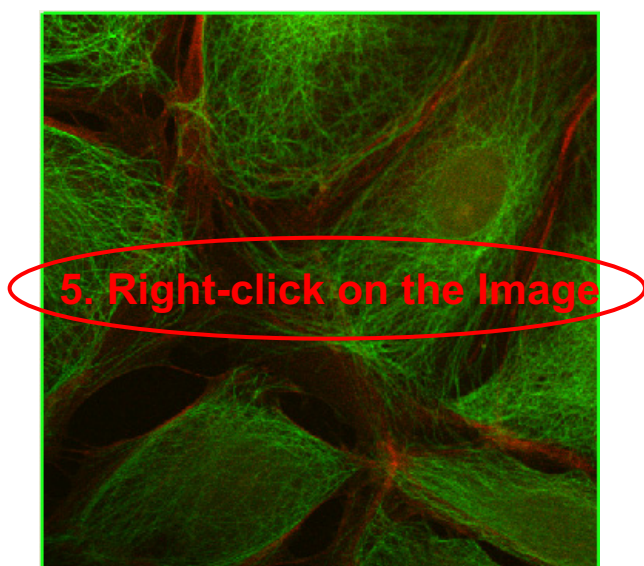
■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)



1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.
2. Check Sequential and select Line.
3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
4. Press the XY button to acquire an image.
5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.) The image is acquired.



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

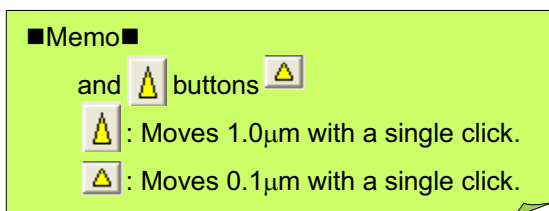
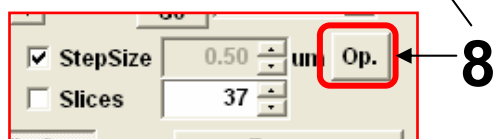
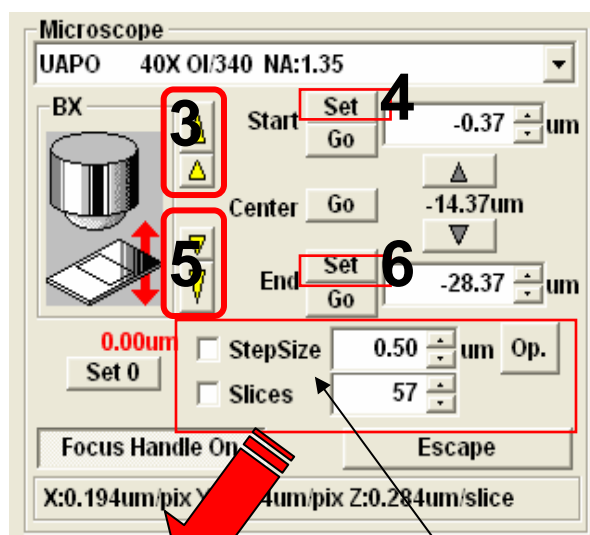
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Double Stain on XYZ Image)

■ Acquisition of 3D images (XYZ) (fluorescence image only) ■

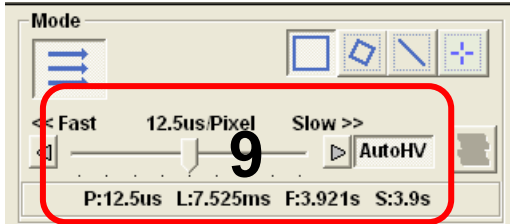
Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



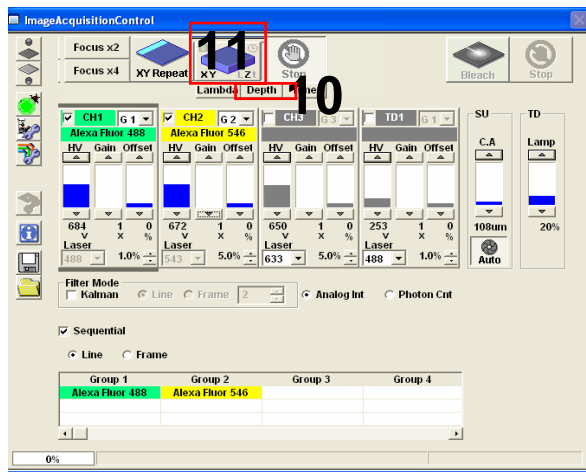
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

Image Acquisition (Double Stain on XYZ Image)



9. Select AutoHV and then select ScanSpeed.

10. Select Depth.



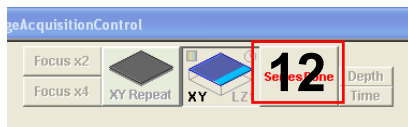
11. Press the XYZ button to acquire an image.

12. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:

Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

13. Right-click on the Image

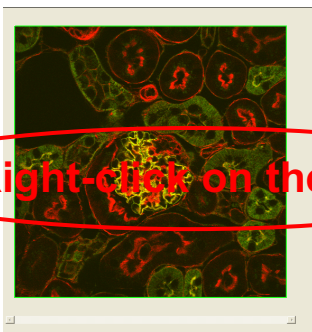


Image Acquisition (Four Stain on XY Image)

■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■

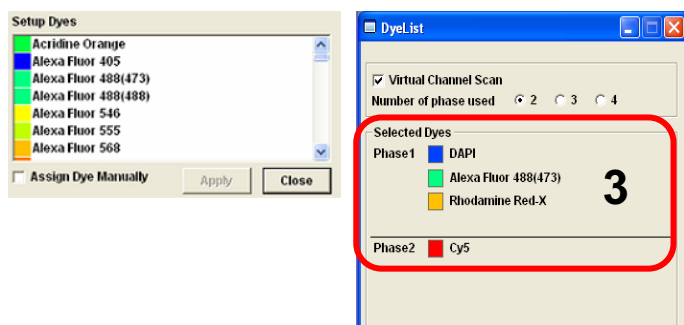
Sample: Four stain of Blue fluorescence dye (DAPI), green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

This is the procedure to acquire images through Virtual Channel scan

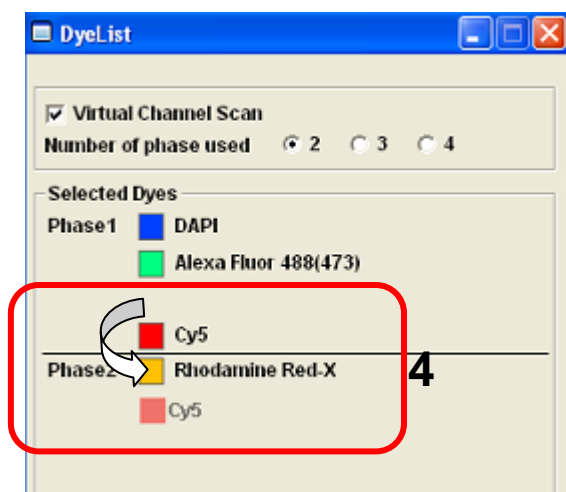


1. ☒ Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then **“Virtual Channel Controller”** is automatically turned on.

2. Select a number of Virtual Channel from **“Number of phase used”**.



3. Select 4dyes from DyeList 4th dye is registered in **“the Phase 2”**.



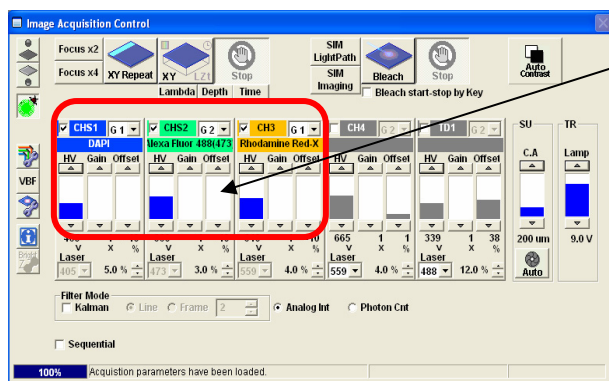
* RodaminRed is able to be registered on **“Phase2”** to drag.

Image Acquisition (Four Stain on XY Image)



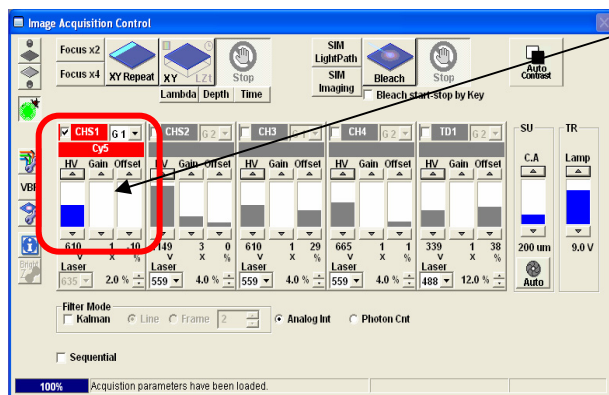
4. Select "Phase1", "DAPI", Alexa488, RhodaminRed are registered on ImageAquisitionControl.

* Slit and Filter, DM are automatically set for "DAPI", "Alexa488" or "PhodaminRed"



5. Select "Phase2", Cy5 is registered on ImageAquisitionControl

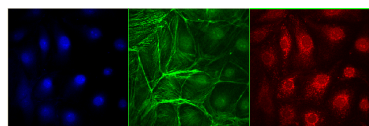
* Slit and Filter, DM are automatically set for "Cy5"



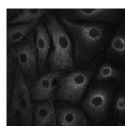
Adjust the image at each phases


"Phase1"

"Phase2"



6




6. Adjust the image to click  "XY Repeat" at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

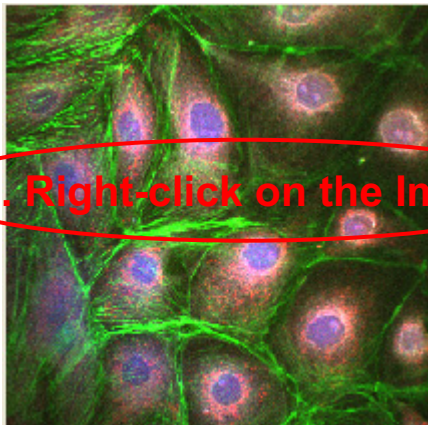
Image Acquisition (Four Stain on XY Image)



7

7. Click  on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type “ oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

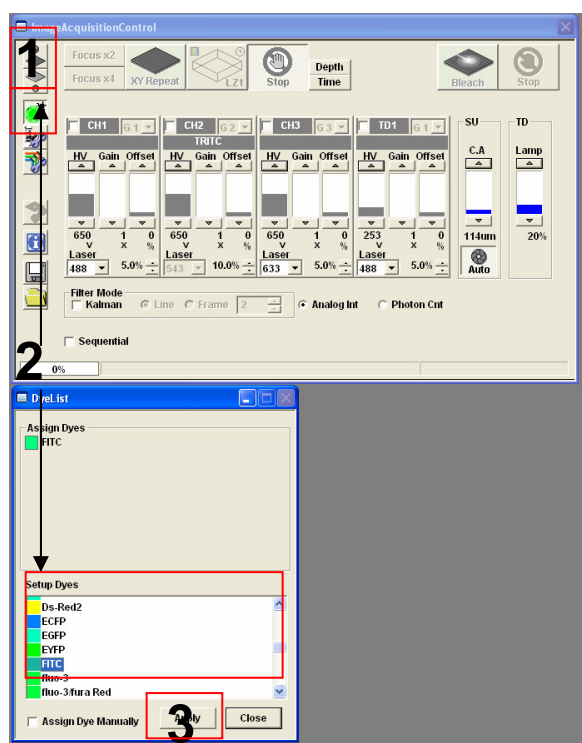
OIB format:



Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image

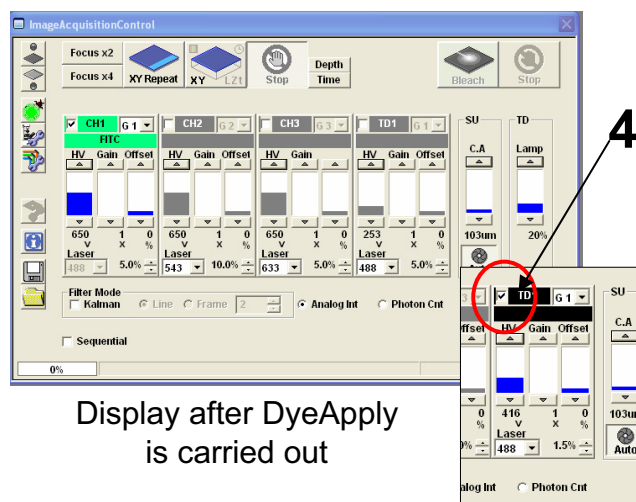


1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

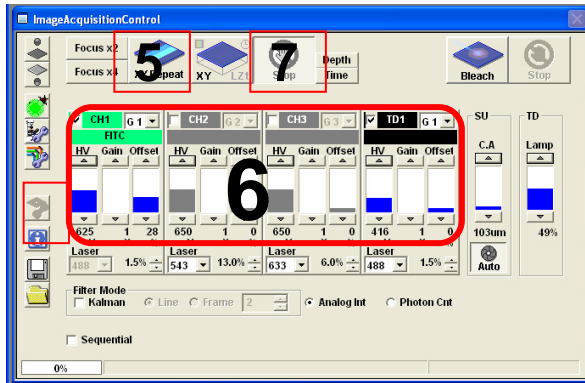
3. Click on the Apply button.
(The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

4. Check TD1.

Image Acquisition (Single Stain + DIC on XY Image)



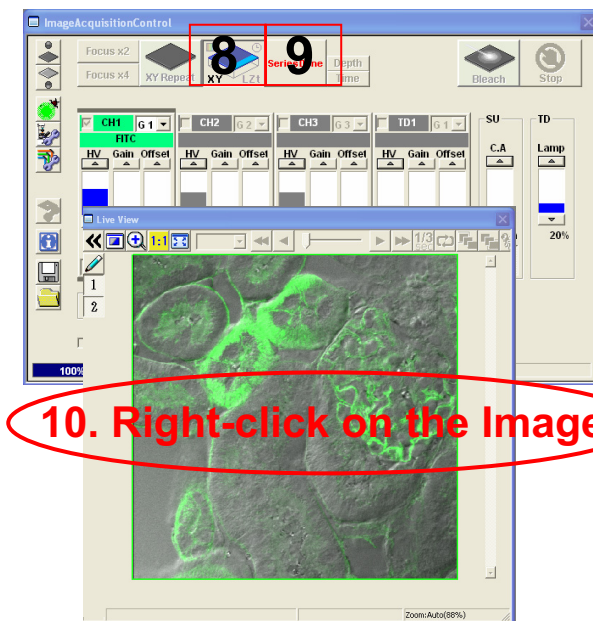
5. Press the “XY Repeat” button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the “Stop button” to stop scanning.

8. Press the “XY button” to acquire an image.

9. Click on “SeriesDone”, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

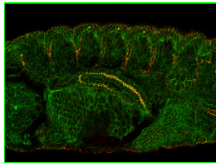
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

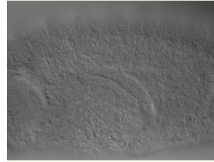
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Merge the images between fluorescent XY image and DIC image

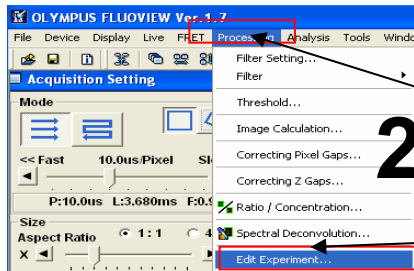
Edit different each files to the same file. This is available for making merge image
Between fluorescent image and focused DIC image.



1

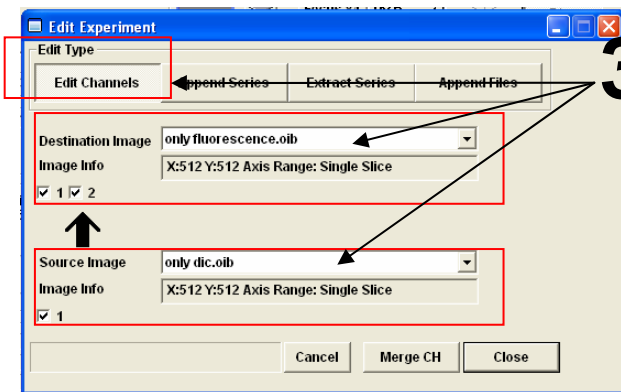


1. Open fluorescent image and
DIC image.



2

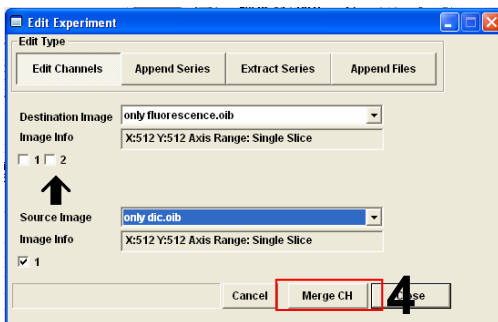
2. Select
Edit experiment from **Processing**



3

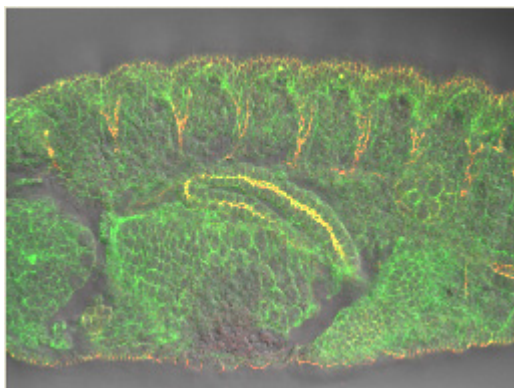
3. Click **Edit Channels**, and select
fluorescent image file at
Destination Image, select DIC
image at **Source Image**.

* Check Image Info ☒ 1 ☒ 2 to make the.
merge file. If all channels are checked, all
channels are reflected in the new merged file.



4

4. Click **Merge CH**, and then
the fluorescent image and the DIC
image are merged as the new file .

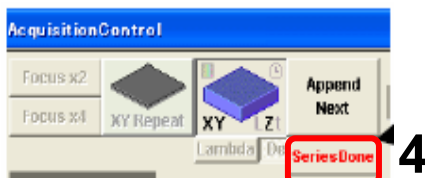
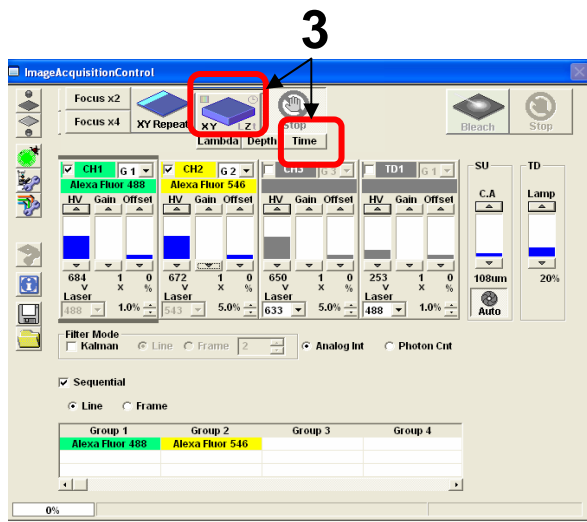
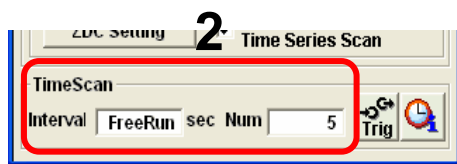


5

5. Merged image between the
fluorescent image and the DIC
image.

Image Acquisition (Single Stain on XYZT Image)

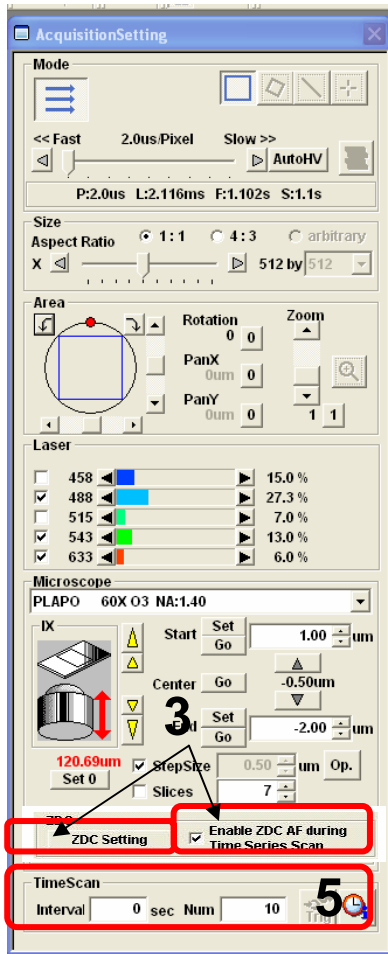
This is available for the Time series scan experiment.



1. Adjust the image.
* Refer P17,18
2. Enter interval time to "Interval"
Enter interval number to "Num"

Example: Acquiring time series scan images every 5minutes for 1hour is below,
3. Select "Time" and then click XYTbutton to acquire Time series scan image.
4. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

Image Acquisition (Single Stain on XYZT Image)



1. Adjust the image.

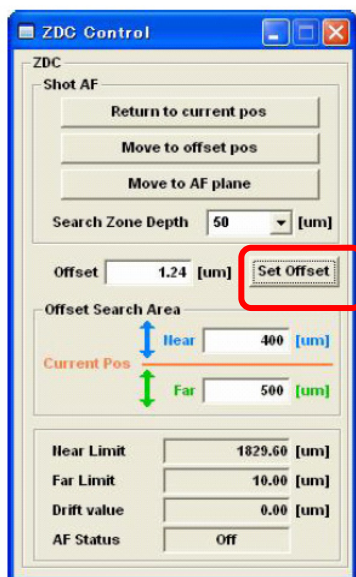
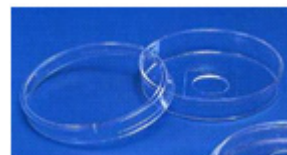
* Refer P17,18

2. Insert ZDC unit to left side.

3. Check “EnableZDC AF during Time Series Scan” ☒ and click “ZDC setting”.

4. Click “Set Offset” to register auto focus position.

* Note: Have to use glass bottom dish below, otherwise ZDC doesn't work.



5. Set “Interval” and “Num” and then click “XYZT” to acquire the time series image.

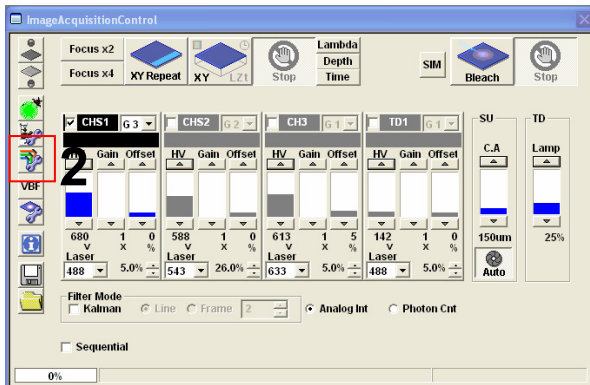
* Note: In case of using ZDC for Time series Scan, follow below limits
Interval number is more than 60 sec,
Rest Time is more than 30 sec,
otherwise ZDC doesn't work.



* If use “TimeControler”, Time Series Scan is able to done even interval number is within 60sec and Rest Time is within 30sec.

Image Acquisition (Spectral Image on XYL Image)

■ Acquisition of a spectral image (XYL) ■

Sample: Double stain of green fluorescence dye (Alexa Fluor 488)
and green fluorescence dye (YOYO-1)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the  button to view the optical path diagram.

3

3. Make settings as shown below.

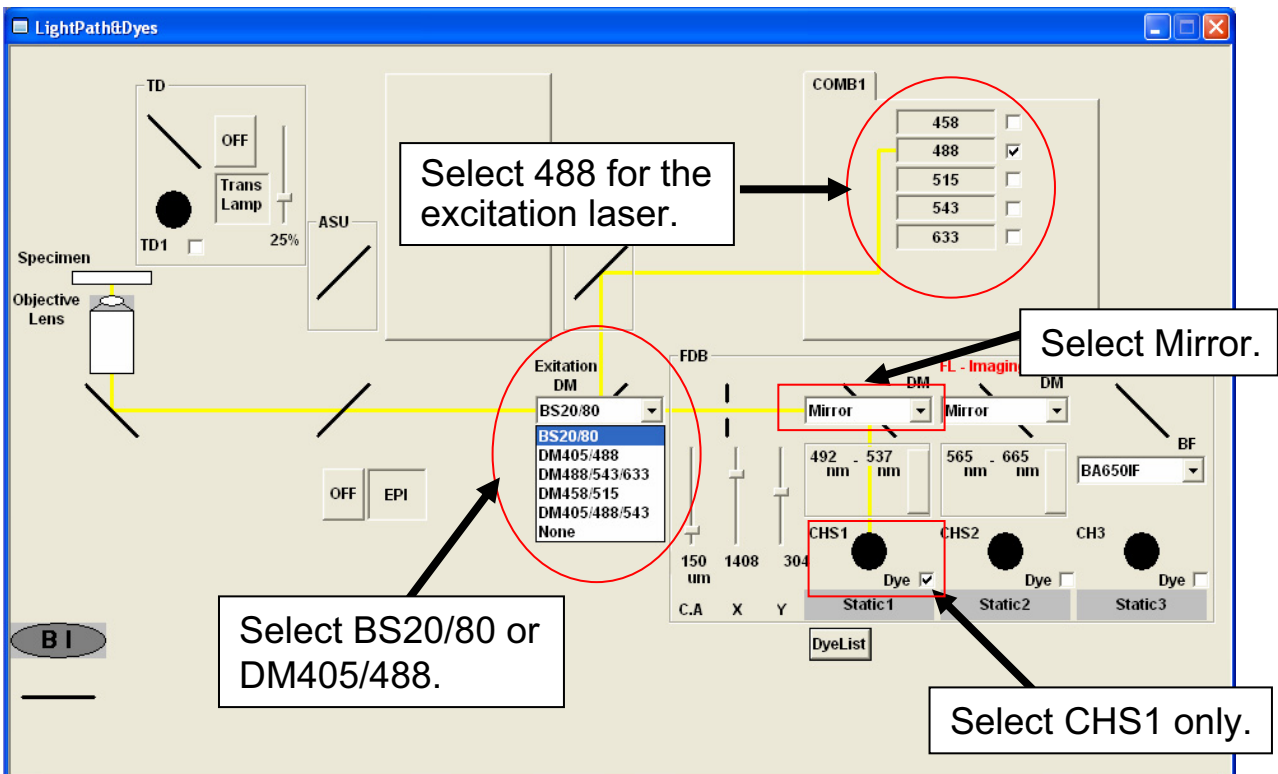
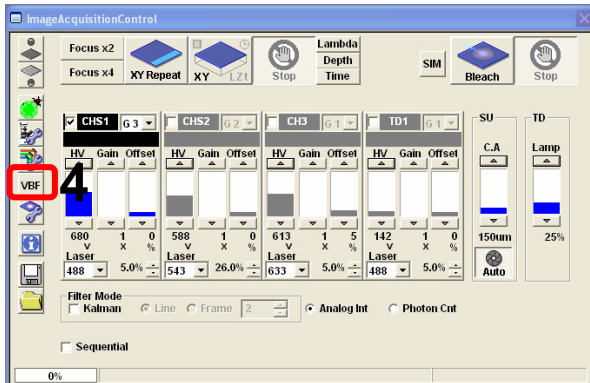
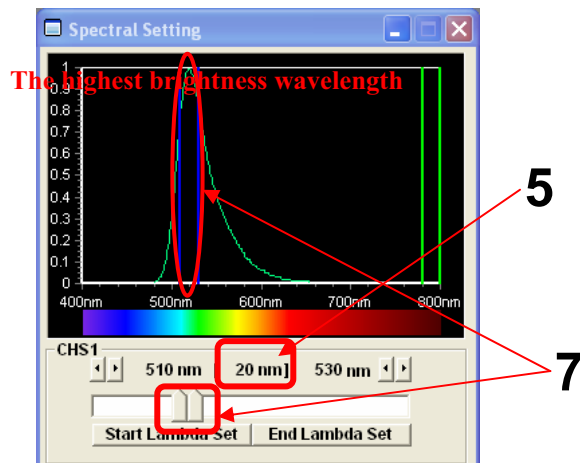


Image Acquisition (Spectral Image on XYL Image)




4. Click on the  button, and the Spectral Setting window appears.

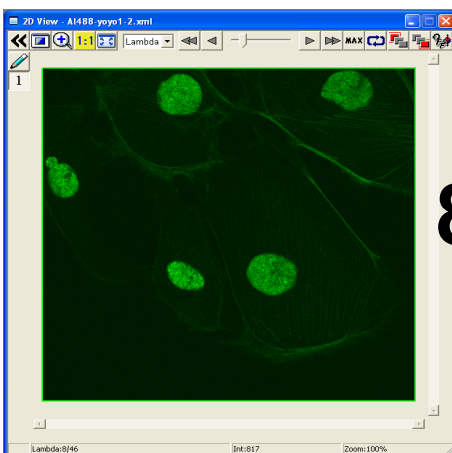
5. Set the slit width for CHS1 to 20 nm, for example.



6. Press the XY Repeat button to start scanning.

7. While observing the image, Click the left side of slit  and drag to the point which the highest brightness is achieved.

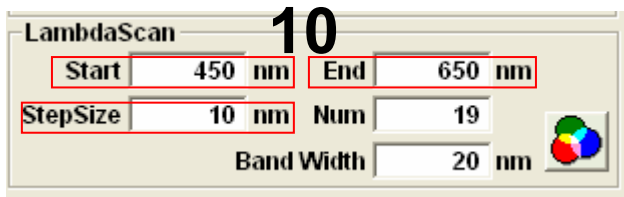
Note: Move the slit position only while keeping the slit width at 20 nm.



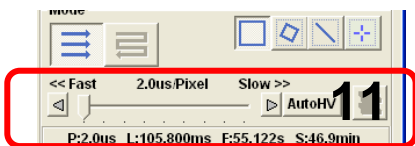
8. Adjust the image on the highest brightness.

9. Press the Stop button to stop scanning.

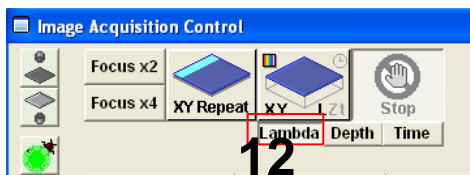
Image Acquisition (Spectral Image on XYL Image)




10. Set the range of wavelength to be acquired, the slit width and the step.
- Start = Start wavelength
 - End = End wavelength
 - Resolution = Slit width
 - StepSize = Step

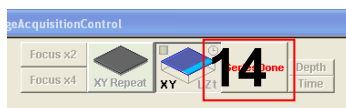


11. Select AutoHV and then select ScanSpeed.
- *As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



12. Select Lambda.

13.  Press the XYZ button to acquire an image.



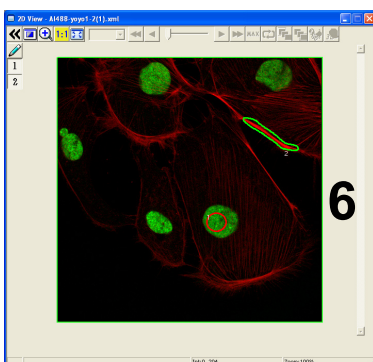
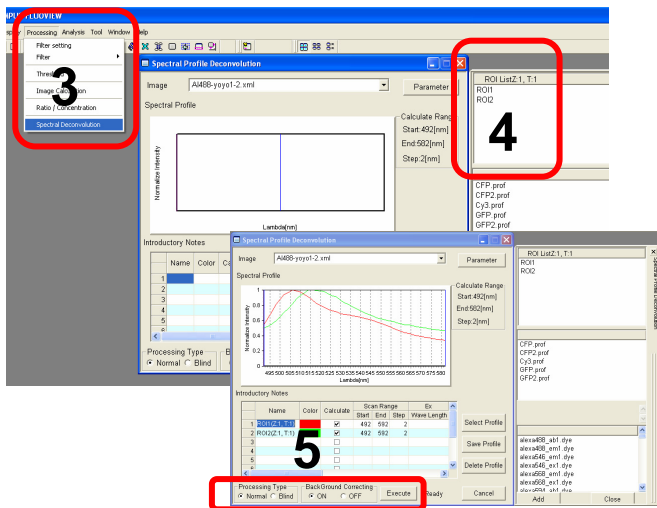
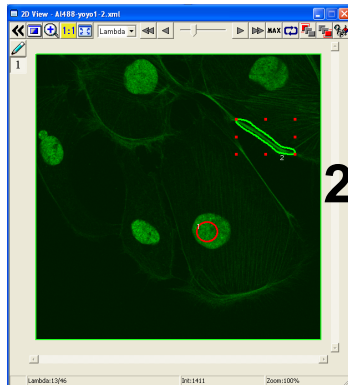
14. Click on SeriesDone, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

Image Analysis (Unmixing)

I. When each fluorescence dye point is clear

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, derive the fluorescence spectrum for each fluorescence dye and obtain an unmixed image based on the fluorescence spectrums.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



Unmixed image

1. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.
2. Enclose a point dyed with Alexa Fluor 488 only and a point dyed with YOYO1 only.
3. From Processing on the menu bar, select Spectral Deconvolution.
4. Double-click on ROI1 and ROI2.
5. Check that the Processing Type is set to "Normal" and click on Execute.
6. An unmixed image is obtained.

Indicates channel assignments of unmixed images

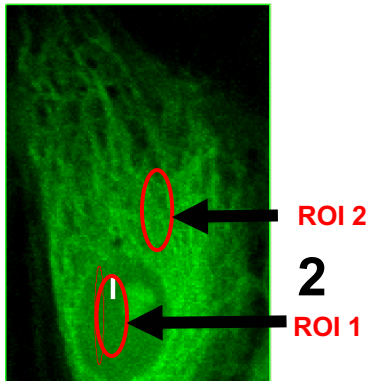
Introductory Notes				
	Name	Color	Calculate	
1	ROI1(Z:1, T:1)	Red	<input checked="" type="checkbox"/>	8
2	ROI2(Z:1, T:1)	Green	<input checked="" type="checkbox"/>	
3			<input type="checkbox"/>	
4			<input type="checkbox"/>	

Image Analysis (Unmixing)

I. When each fluorescence dye point is clear

Sample: single stain of green fluorescence dye (GFP)
and auto fluorescence from cell

1



1. Open the XYL image (GFP + auto fluorescence).

2. Enclose a point dyed with GFP only and a point dyed with auto fluorescence only.

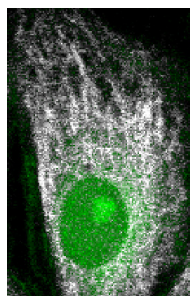
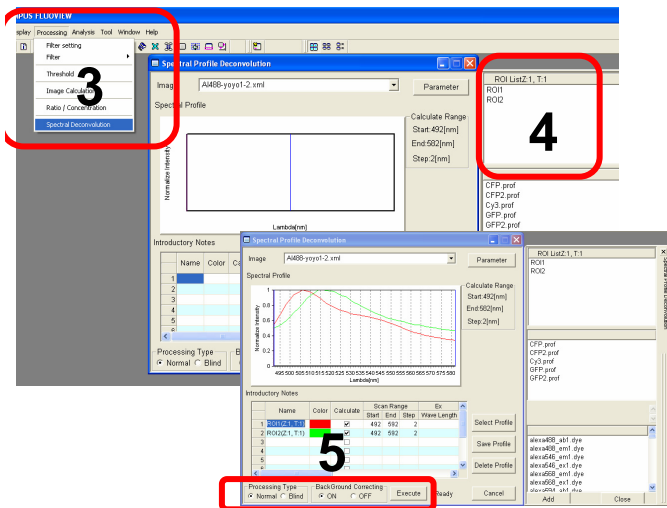
3. From Processing on the menu bar, select Spectral Deconvolution.

4. Double-click on ROI1(GFP) and ROI2(Auto fluorescence).

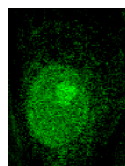
5. Check that the Processing Type is set to "Normal" and click on Execute .

6. An unmixing image is obtained.

Green color is GFP.
Gray color is Auto fluorescence.



6



Only GFP image

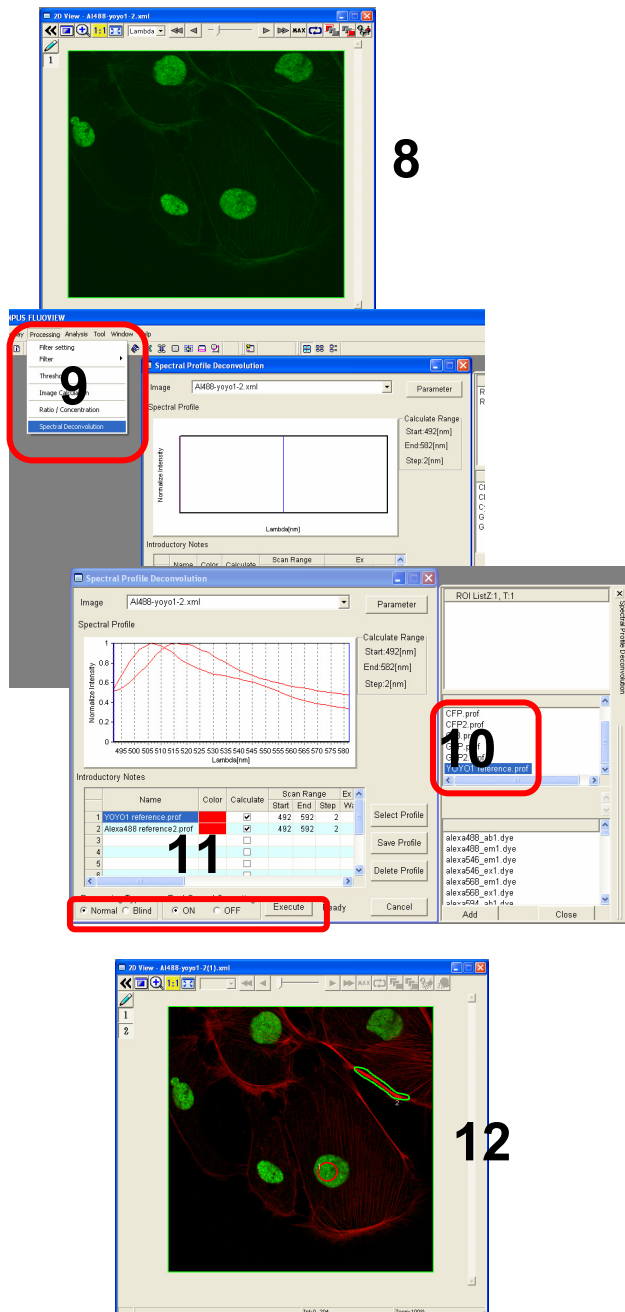
Unmixing image between GFP and Auto fluorescence

Image Analysis (Unmixing)

II. When a control sample is used

From an XYL image with a single type of fluorescence dye, derive the fluorescence spectrum of the dye and obtain an unmixed image based on the fluorescence spectrum.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



8. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.

9. From Processing on the menu bar, select Spectral Deconvolution.

10. Double-click on Alexa Fluor 488 and YOYO1 (which have been registered) in the database of fluorescence spectrums.

11. Check that the Processing Type is set to "Normal" and click on Execute.

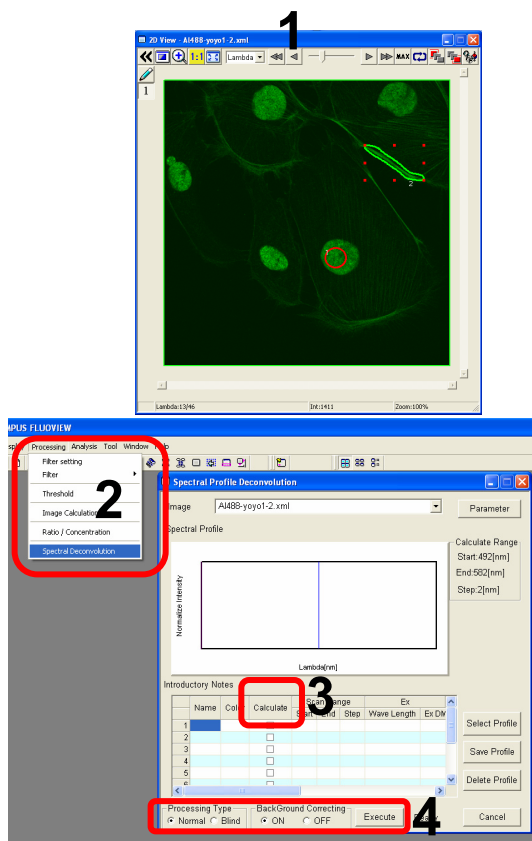
12. An unmixed image is obtained.

Image Analysis (Unmixing)

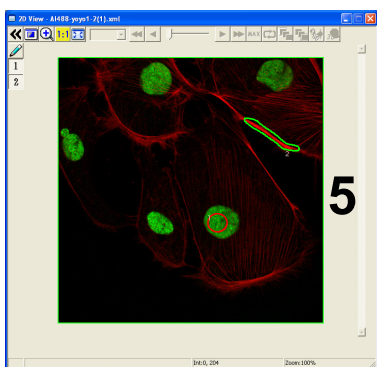
III. When only the number of types of fluorescence dyes is known (Blind Unmixing)

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, obtain an unmixed image based on only the number of types of fluorescence dyes.

Sample: Sample with two unknown types of fluorescence dyes



1. Open an XYL image file for a sample that has two unknown types of fluorescence dyes.
2. From Processing on the menu bar, select Spectral Deconvolution.
3. Click on two Calculate check boxes. (Click on three boxes when three types of fluorescence dyes are used.)
4. Check that Processing Type is set to "Blind" and click on Execute.

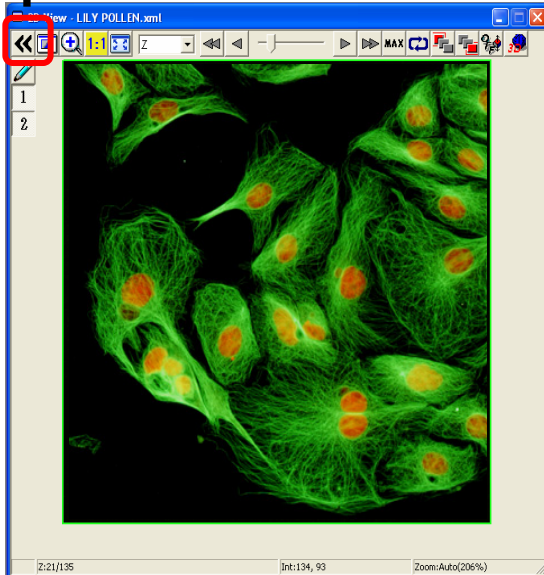


Unmixed image

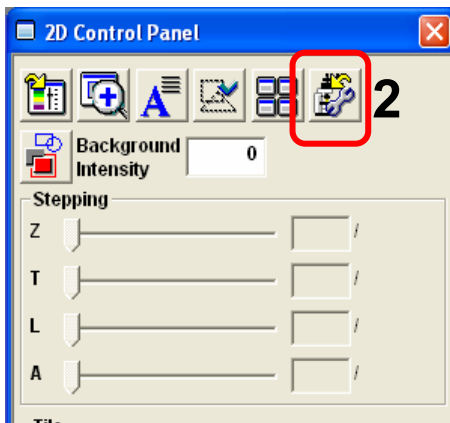
5. An unmixed image is obtained.

Reload the image conditions

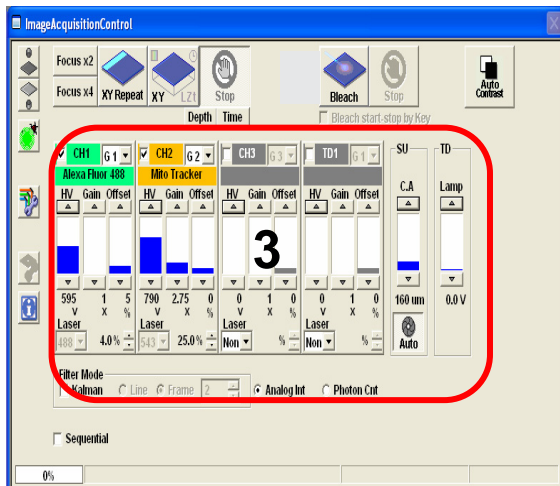
1



1. Open the file and click



2. Click



3. The conditions (HV,Offset, CA and so on) are reloaded .

Overview of the 2D Operation Panel

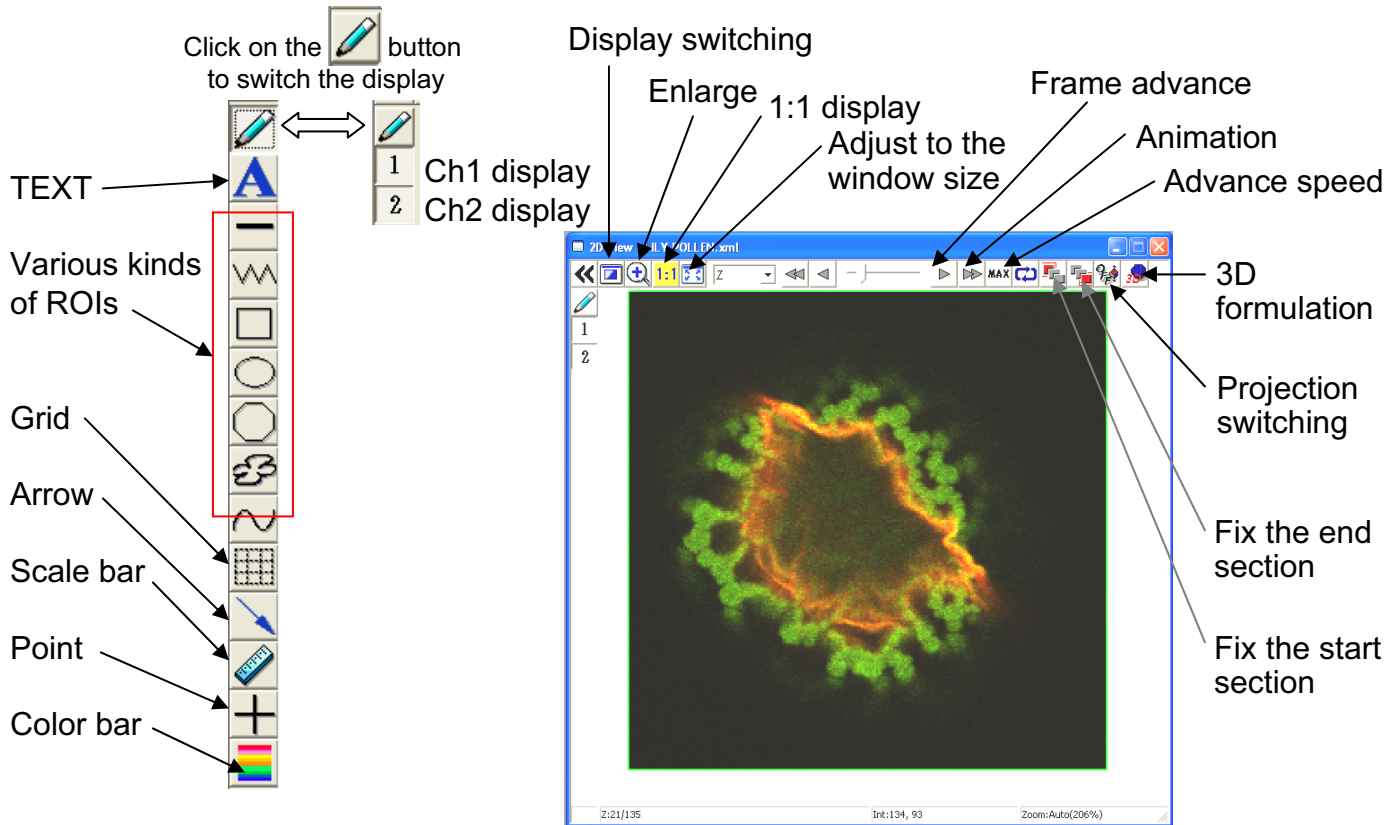
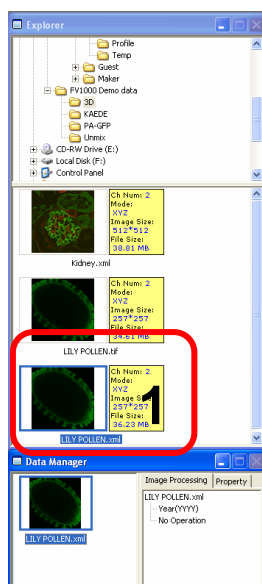
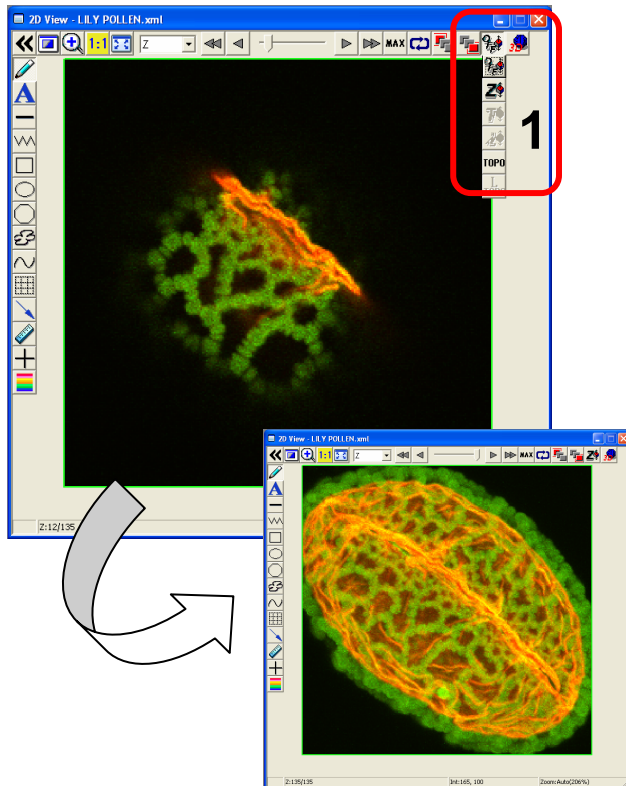


Image Analysis (Opening a File)

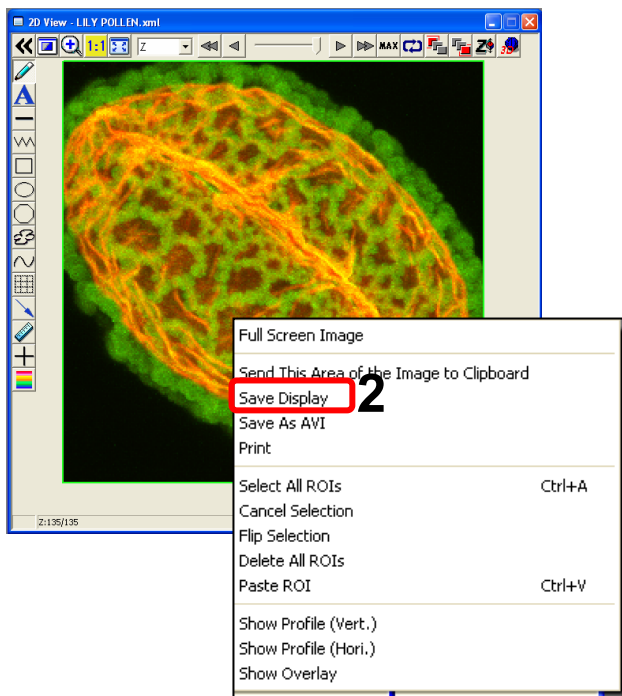


1. Double-click on a file to be opened from Explorer.

Image Analysis (Acquire a Projection Images)



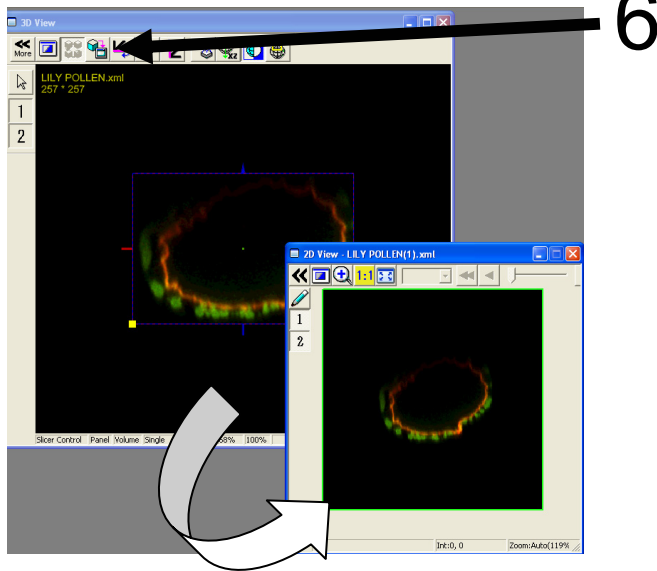
1. Click on the  button to select .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

Image Analysis

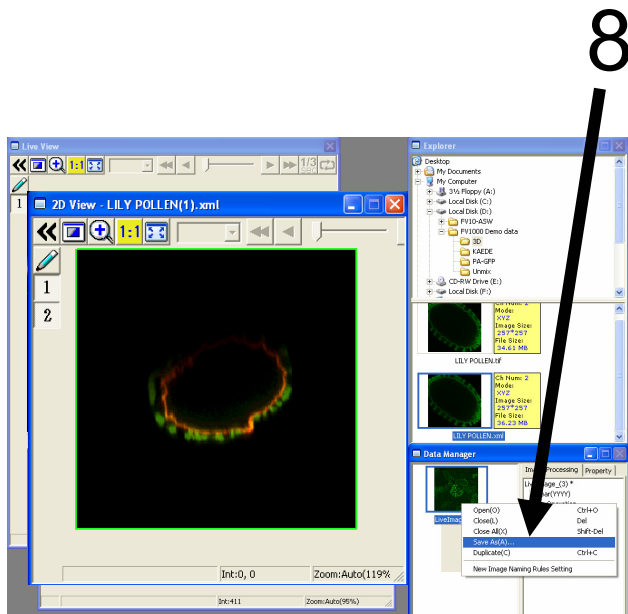
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

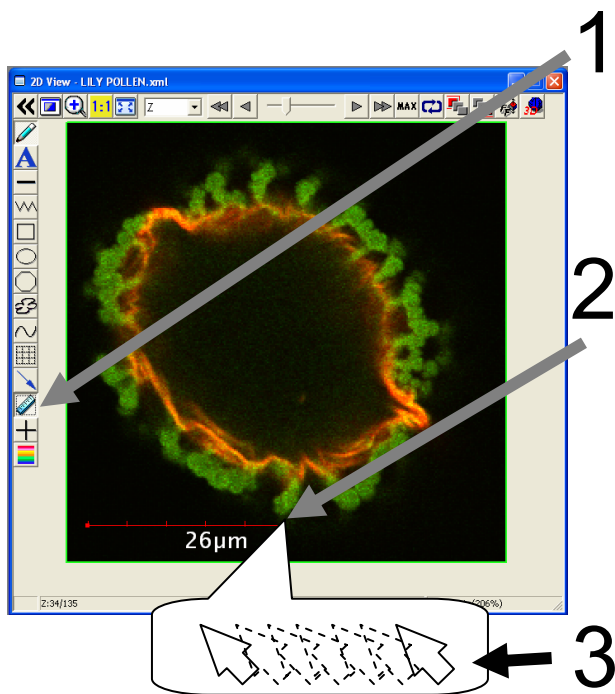
OIF format:


Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Analysis (Inserting the Scale Bar)

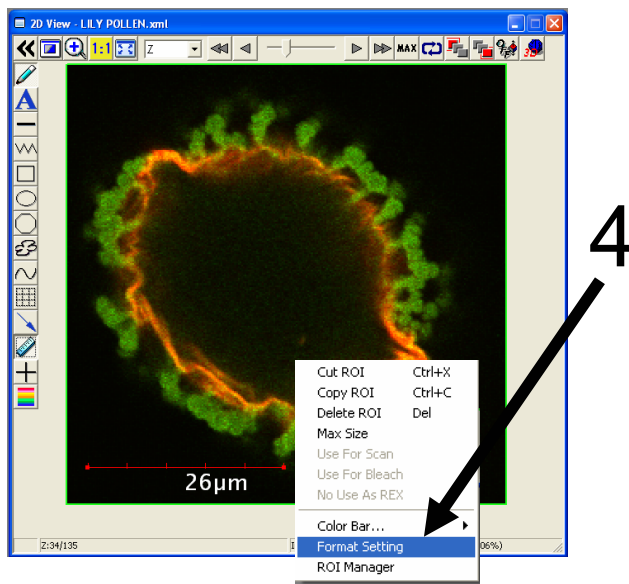


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

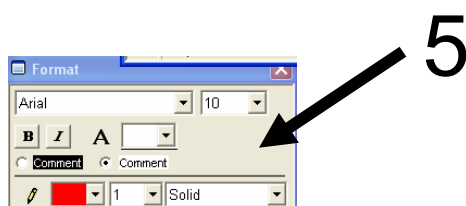
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

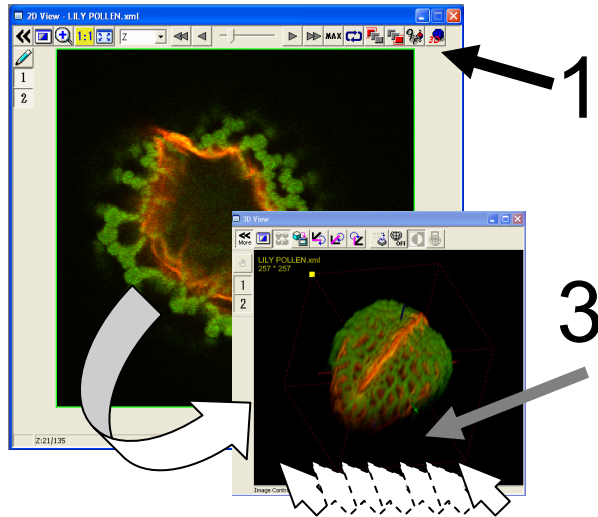
4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

Image Analysis

(Rotating a Three-dimensional Image)





1. Click on the  button for a 2D View-(file name) image.


2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.

Simple animation

4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

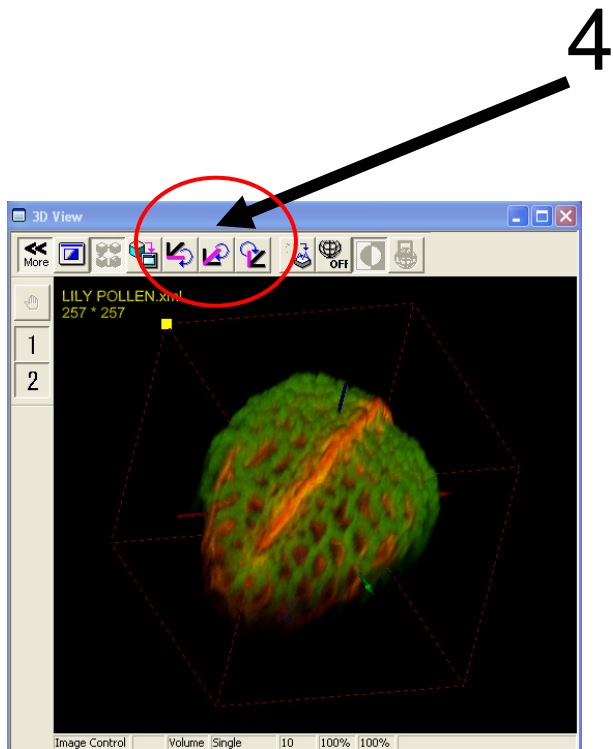
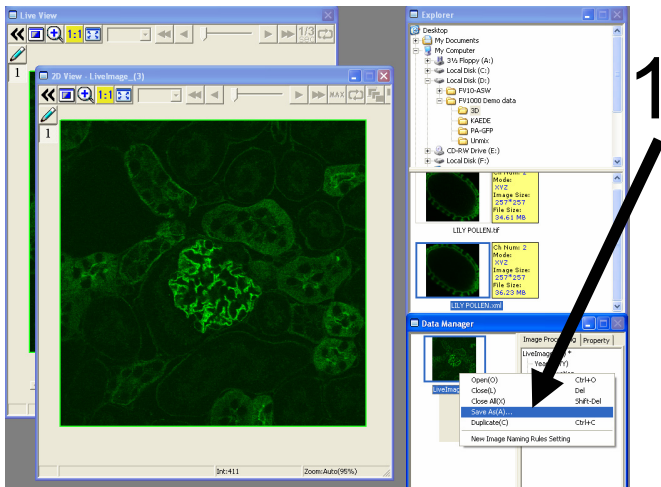


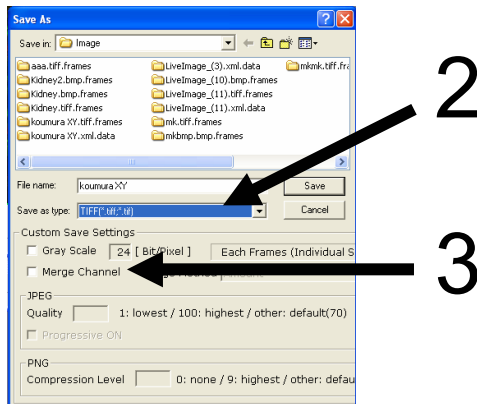
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

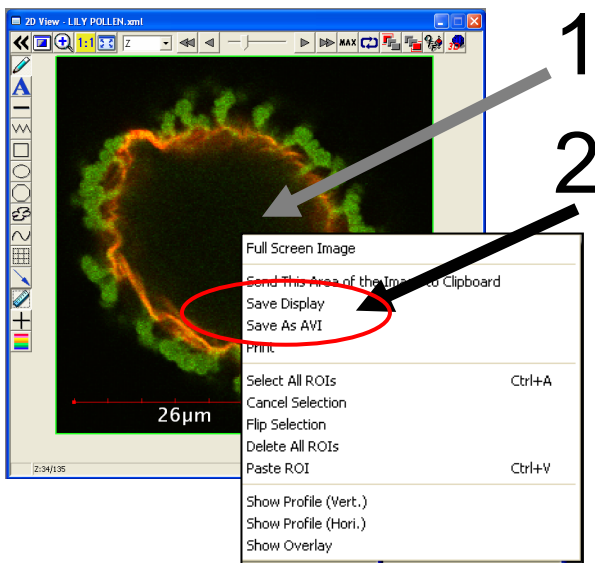
* BMP and JPEG formats are also selectable.



Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.



Convert an image with the scale bar inserted into a BMP format

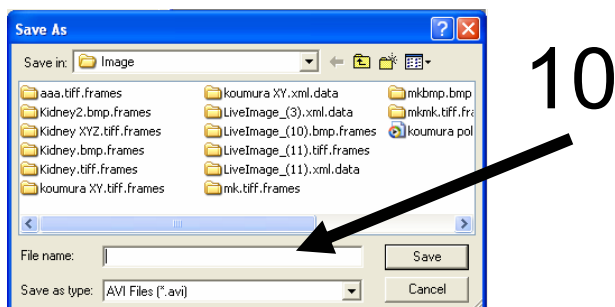
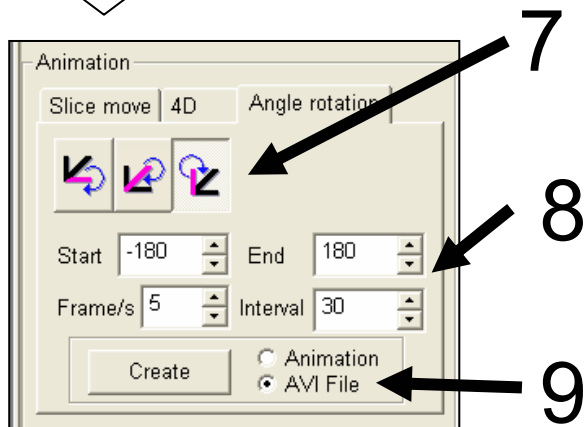
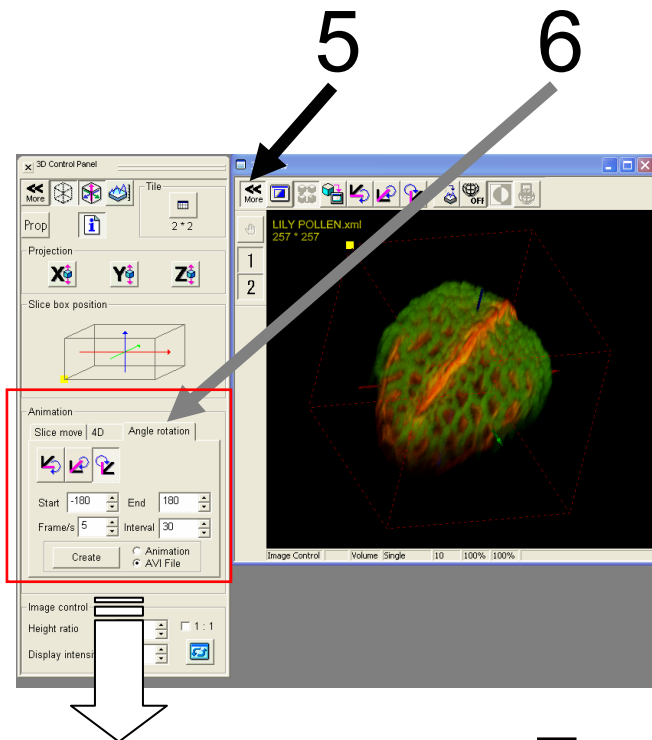
1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

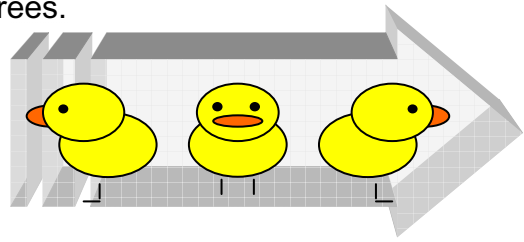
Image Analysis

(Rotating a Three-dimensional animation)



To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the  button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

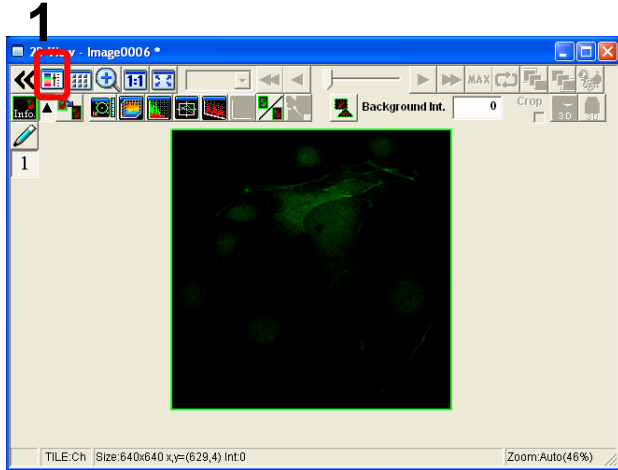
8. Enter the rotation angle.

Start = Angle to start rotation
End = Angle to stop rotation
Frame/s = Rotation speed
Interval = Degrees to be rotated at a time

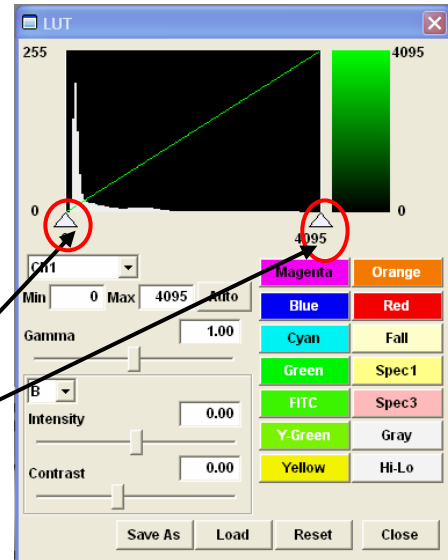
9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

2D Image Analysis (Edit the image color and contrast)




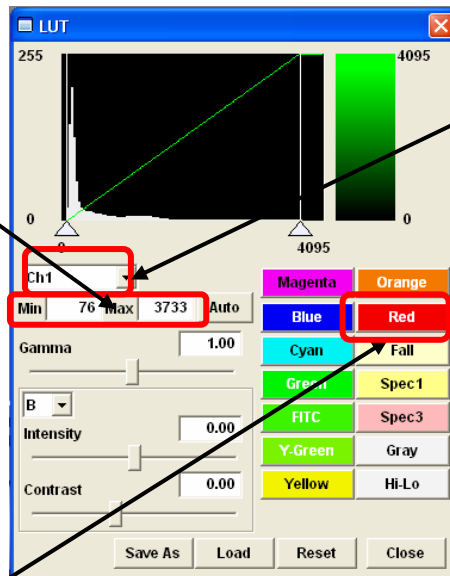
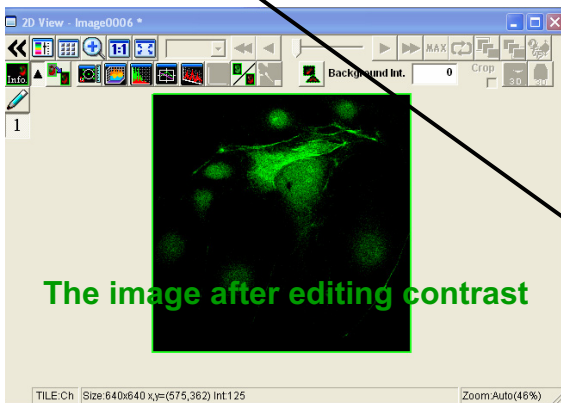
1. Click  "LUT" and then LUT table appears below,



3. Min and Max value are changed and contrast of image is edited.

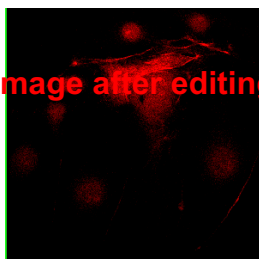
* According to get Min value up , be able to reduce noise of the image.

2.Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min"(Max4095, Min0)



Edit each Ch

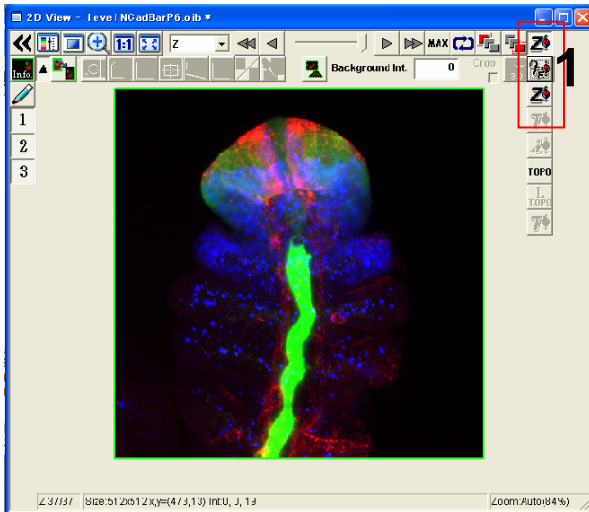
The image after editing color





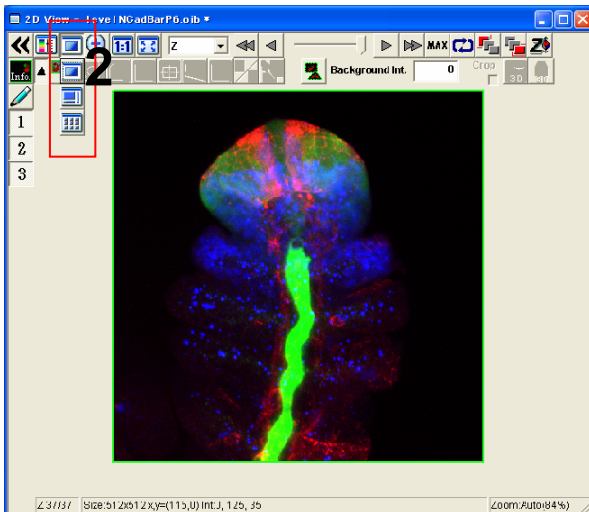
4. To click another color, be able to Edit a color.
Above example: Change Green to Red to click



Red

2D Image Analysis (the image of Z section)

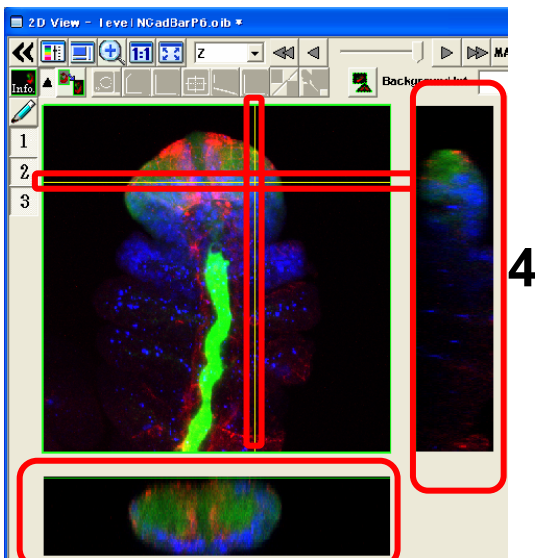


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select .

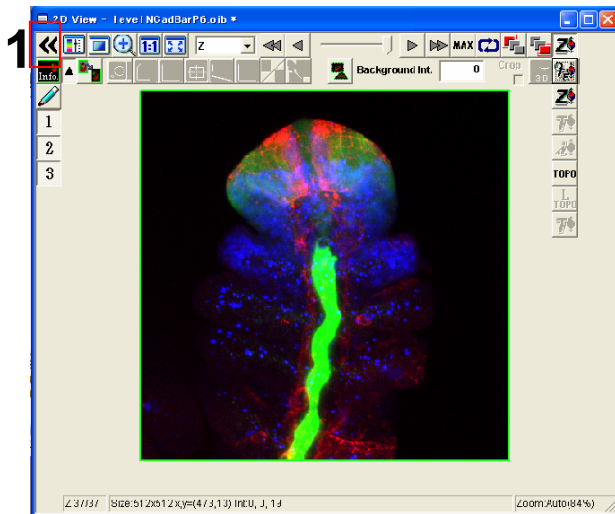
3. The images of Z section is shown on X axis and Y axis.
According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.




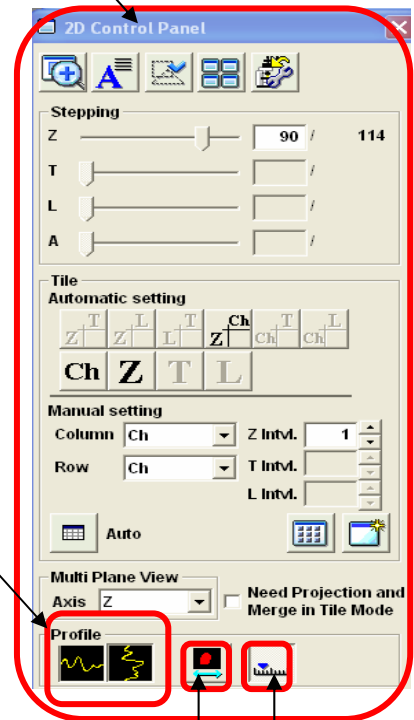
4. The image of Z section on Y axis.

5. The image of Z section on X axis.

2D Image Analysis (Intensity Profile of each Z sections)

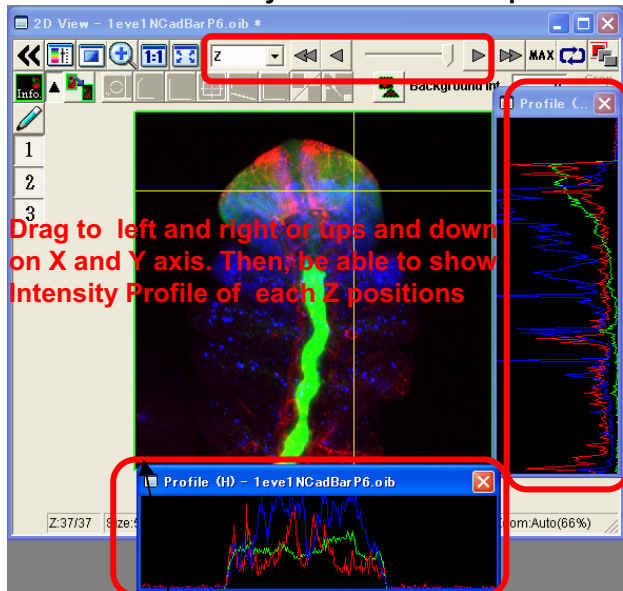


1. Click  and then 2D Control Panel is shown below,




2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

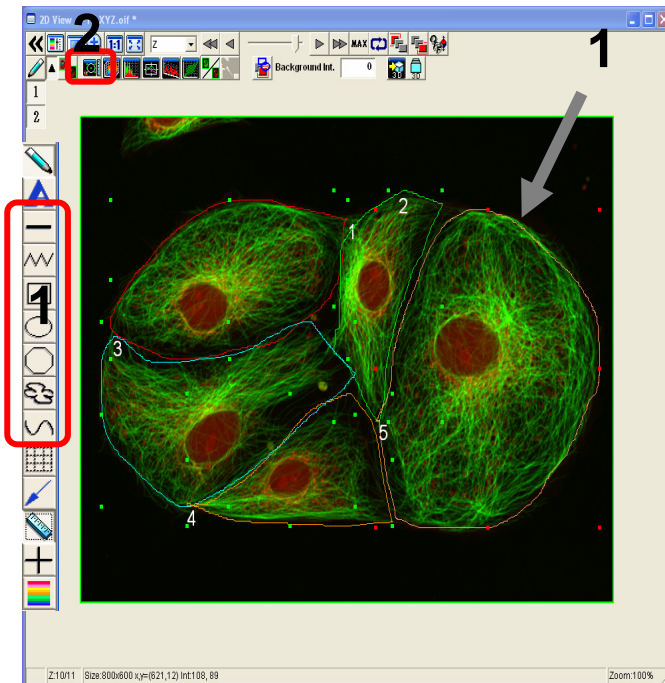
To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click , be able to show as equal scale of Profile window as 2Dimage.

2D Image Analysis (Measure)



1. Enclose interesting regions by ROI

Line on interesting positions by ROI

2. Click  "measure".

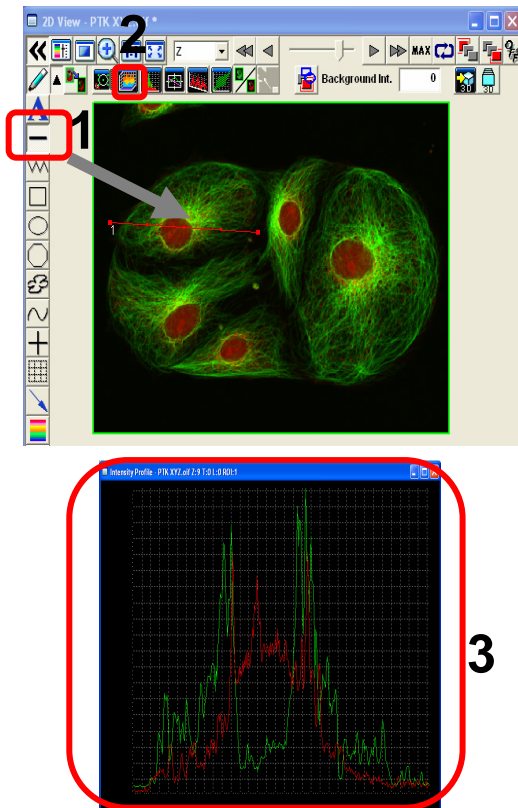
4. According to click "Measure All ROIs", then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs


ROI	CenterX	CenterY	Area	Perimeter	Integration	Average	Max	Min	Range	StdDev	3StdDev	Integration	Average	Max	Min	Range	StdDev	3StdDev
	[um]	[um]	[um*2]	[um]	CHS1	CHS1	CHS1	CHS1	CHS1	CHS1	CHS1	CHS2	CHS2	CHS2	CHS2	CHS2	CHS2	CHS2
1	57.171	48.438	3129.625	241.490	5478264.000	1107.926	4095.000	95.000	4000.000	710.261	2130.783	2952481.000	658.076	3590.000	28.000	3562.000	522.518	1567.554
2	112.522	53.402	1470.188	194.764	9620457.000	1301.724	4095.000	97.000	3998.000	883.602	2650.807	7837013.000	758.280	3468.000	28.000	3440.000	561.877	1685.630
3	51.900	87.103	3274.688	273.215	2573667.000	1003.410	4095.000	94.000	4001.000	700.397	2101.192	9839166.000	569.504	3415.000	53.000	3362.000	443.623	1330.869
4	80.180	111.524	1732.438	211.246	4386227.000	879.766	3836.000	83.000	3753.000	657.656	1972.967	7880740.000	645.072	3380.000	25.000	3355.000	523.061	1569.183
5	150.780	79.732	6120.813	313.258	1878548.000	1244.509	4095.000	96.000	3999.000	725.103	2175.309	4771708.000	559.277	3227.000	41.000	3186.000	439.334	1318.002

2D Image Analysis (Line Intensity Profile on the 2D image)



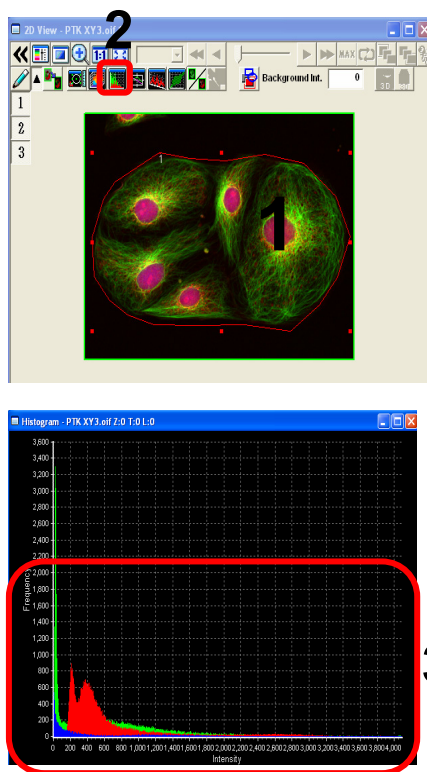
1. Line on the 2D image by ROI



2. Click  "Intensity Profile"

3. "Intensity Profile" on the line is shown as intensity graph .

2D Image Analysis (Histogram)

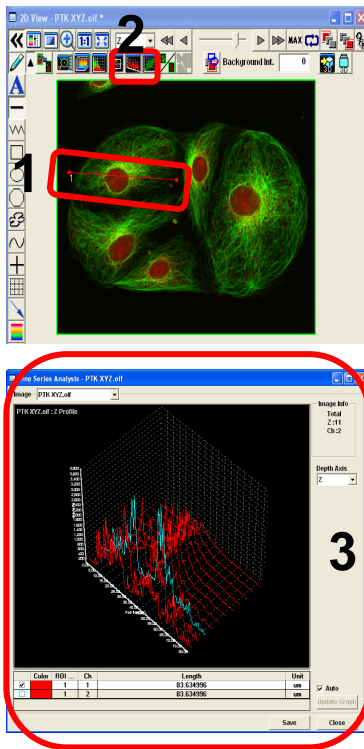


1. Enclose interesting regions by ROI.


2. Click  "Histogram"

3. "Histogram" window is shown as a graph, frequency of intensity of each pixels is plotted on the area enclosed by ROI.

2D Image Analysis (Line Series Analysis)

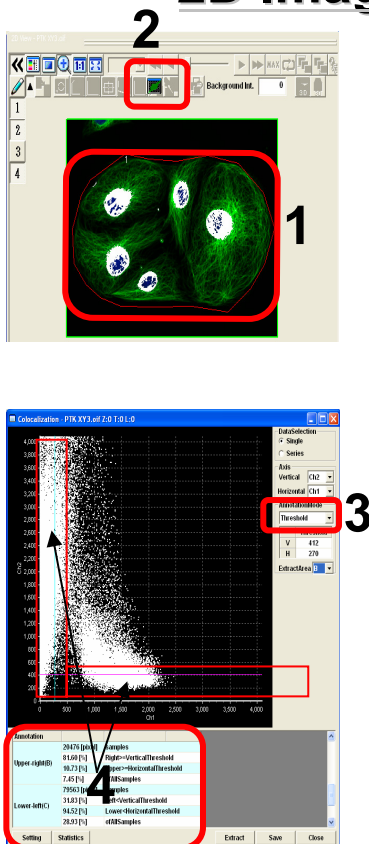


1. Line on the 2D image.

2. Click  "Line Series Analysis"

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



1. Enclose an interesting regions by ROI.

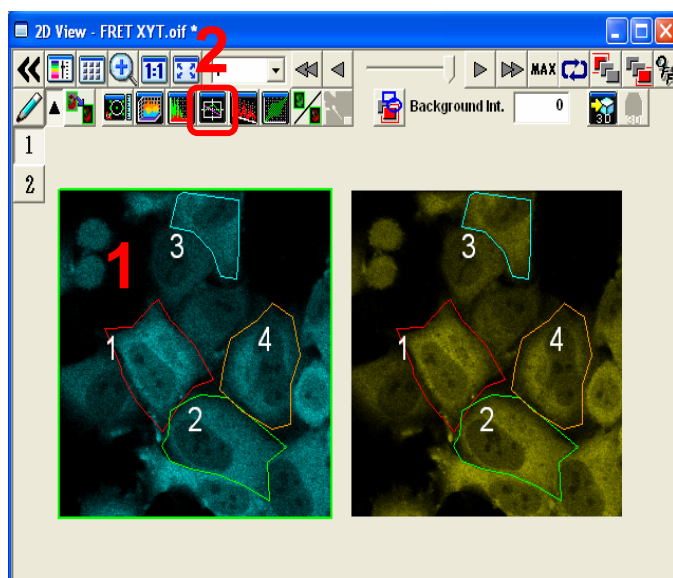
2. Click 

3. Select  **Threshold** from **Annotation Mode**.

4. According to move Thresholds of X,Y axis to right and left ,ups and down (**Enclose red color X,Y axis**), Co-localization result between 2ch is changed .

Information of Co-localization is listed under the scatter plot.

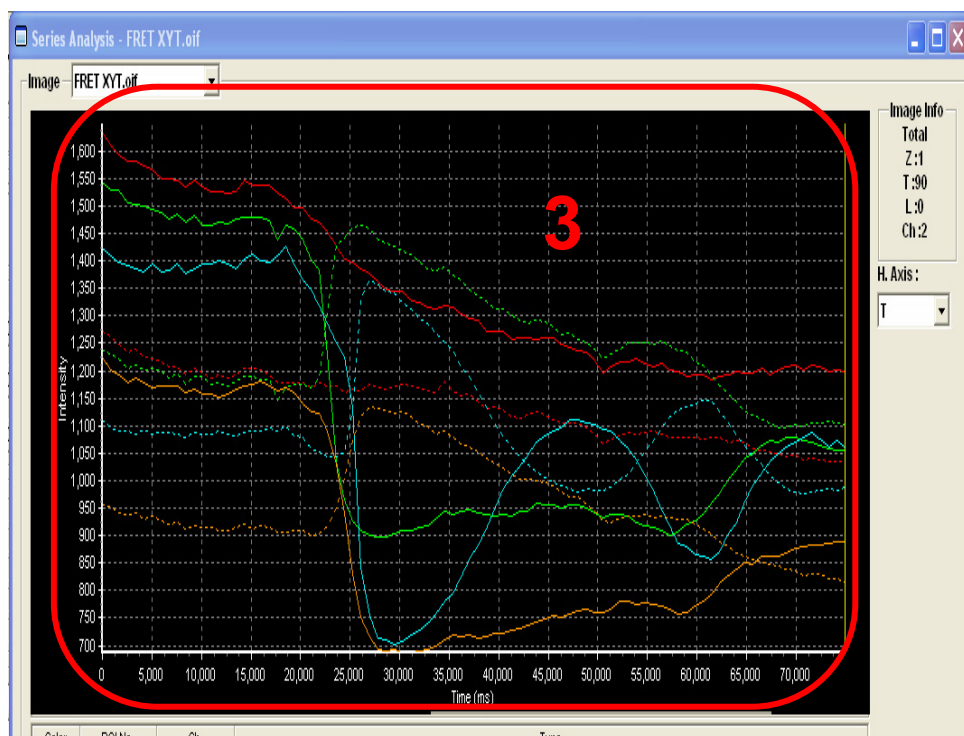
2D image Analysis (Series Analysis TimeLapse)



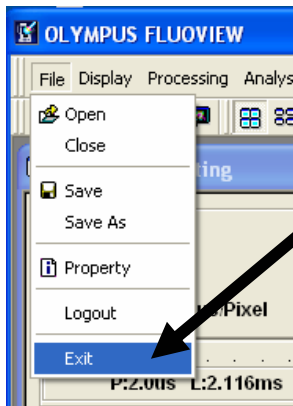
1. Enclose interesting regions by ROI

2. Click  “Series Analysis”

3. “**Series Analysis**” graph is shown below, Y axis shows intensity, X axis shows time and then be able to see time series reaction each ROIs.



Closing the System

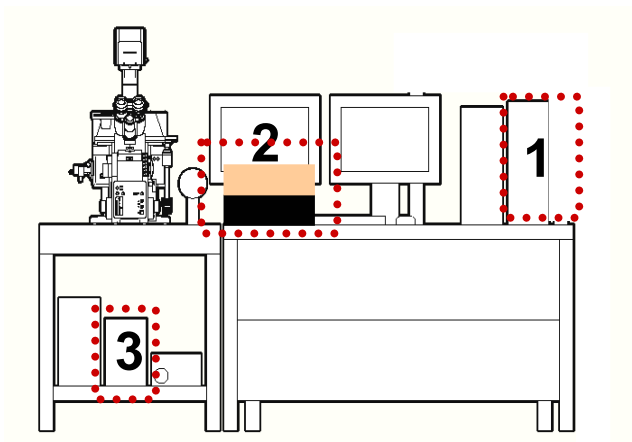


1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.

(2) On the Shut Down Window, select Shut Down and click on OK.



3. Turn the laser OFF.
(Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm)
OFF

3-3. HeNe (G) (543 nm) OFF

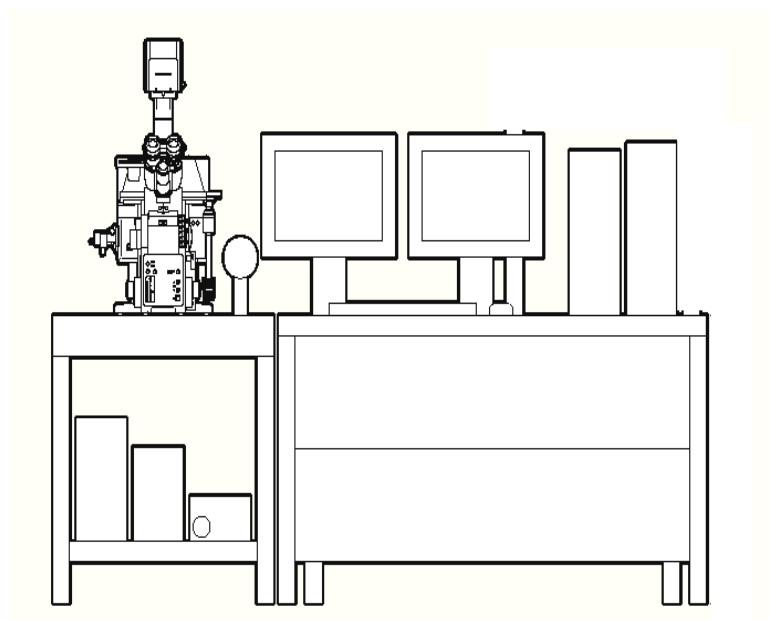
4. Turn the mercury burner power OFF.



Laser Confocal Scanning Microscope

FV1000D Filter Type
(inverted Microscope IX81)

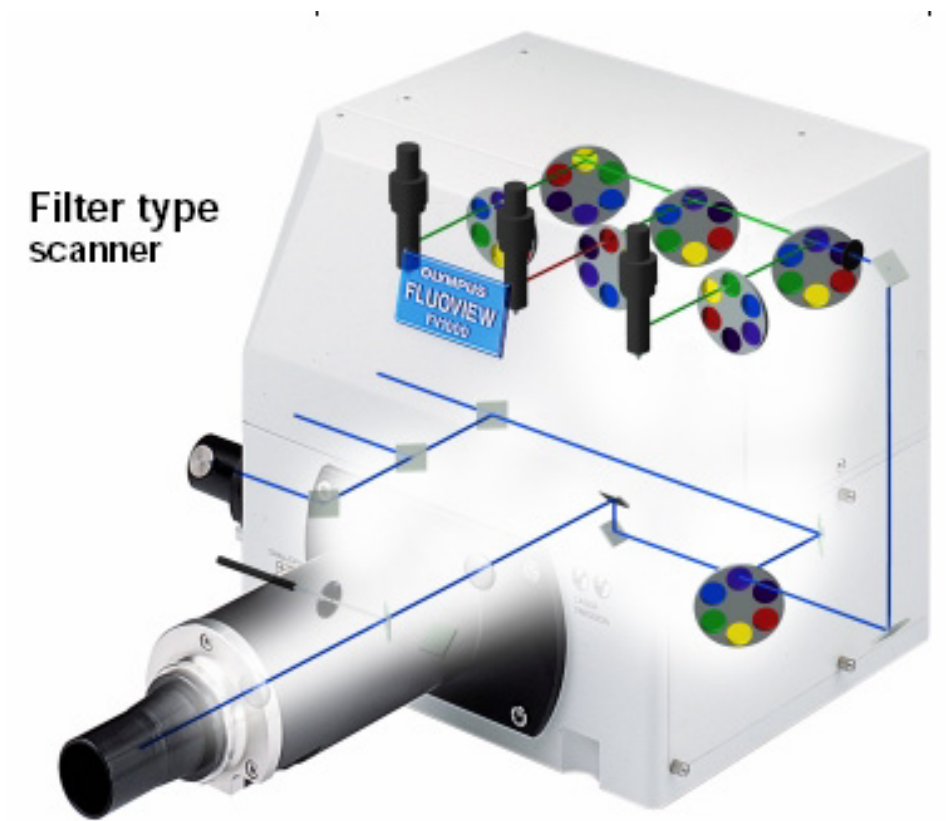
Operation Manual



Contents

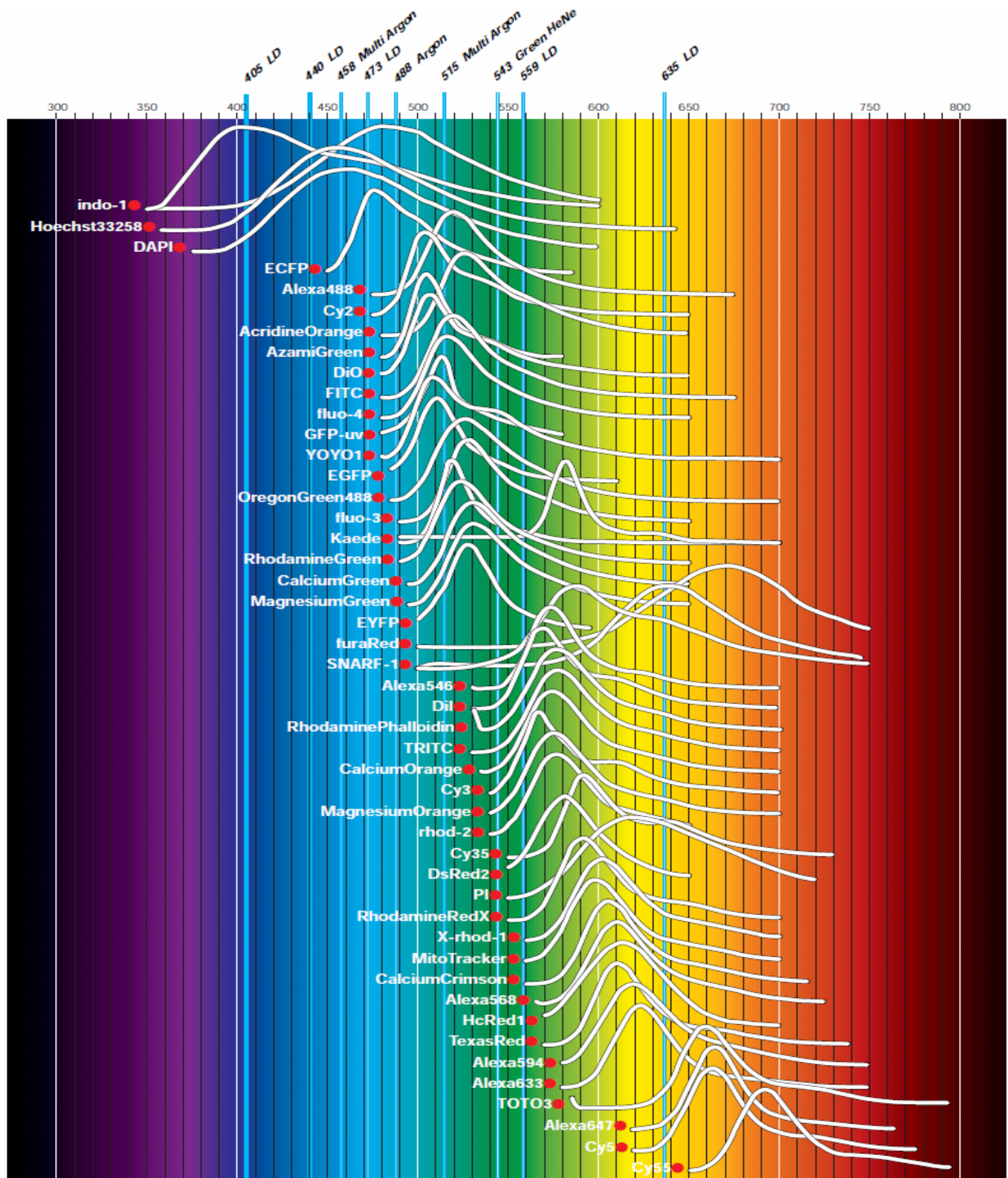
System introduction	-----	3
FV1000D Laser DyeList	-----	4
System Preparation	-----	5
Visible Observation		
Observation of Fluorescence image	-----	6
Observation of Differential Interference Contrast Images	-----	7
Image Acquisition		
Overview of Operation Panel for Image Acquisition	-----	8
Single Stain on XY Image		9-11
Complement of adjusting the image	-----	12-13
Double Stain on XY Image	-----	14-15
Sequential scan Line Sequential	-----	16
Double Stain on XYZ image	-----	17-18
Four Stain on XY Image	-----	19-21
Single Stain + DIC on XY Image	-----	22-23
Merge the image between fluorescent XY image and DIC image	----	24
Single Stain on XYZT Image / ZDC XYZT image	-----	25-26
Reload the image conditions	-----	27
Image Analysis		
Overview of the 2D Operation Panel / Opening a file	-----	28
Making 2D Z projection file Images	-----	29
Saving a Z section image as 2D image	-----	30
Inserting the Scale Bar	-----	31
Rotating a Three-dimensional Image	-----	32
Saving an Image	-----	33
Saving Rotating a Three-dimensional animation file	-----	34
2D Image Analysis		
Edit the image color and contrast	-----	35
The image of Z section	-----	36
Intensity Profile of each Z sections	-----	37
Measure	-----	38
Line Intensity Profile on the 2D image / Histogram	-----	39
Line Series Analysis / Co-localization	-----	40
TimeLapse Analysis	-----	41
Closing the System	-----	41

Filter Type Main Scanner

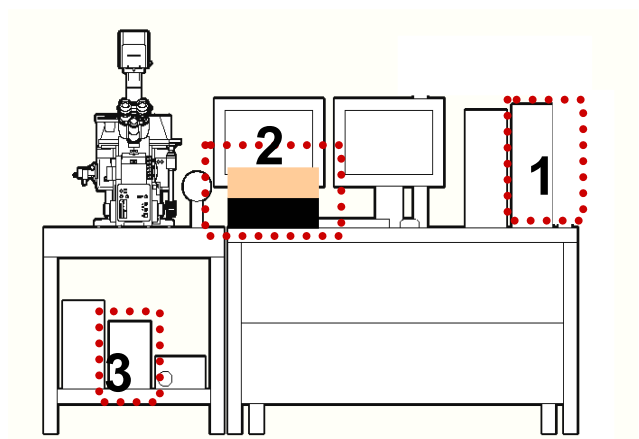


Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe (G) 543nm



System Preparation



1. Turn the computer ON.
[In case of equipped concentrated power supply, power on it first]
2. Turn the laser ON
(Turning the key switch)
 - 2-1. LD559nm ON
 - 2-2. Multi Ar 458nm 488nm 515nm
 - 2-2. HeNe(G) (643nm) ON
3. Turn the mercury burner ON for Fluorescence observation.




Wait for a moment until the software is started

4. Log on Windows

Enter Password ,Customer name is below

User name: Administrator

Password : fluoview

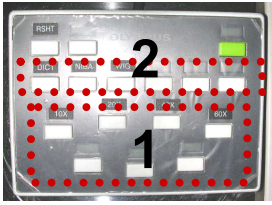
5.  Double click this icon to log on to ASW

User name: Administrator

Password : Administrator

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch




1. Select an objective lens by using the hand switch
2. Select fluorescent filter cube

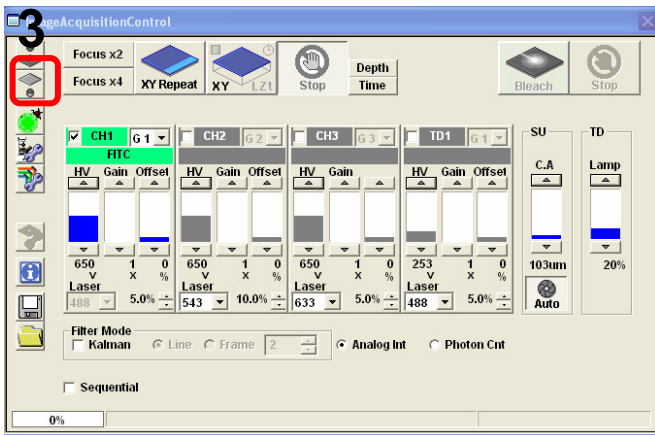
MEMO

Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.: FITC, EGFP)

WIG: Green Excitation / Red Fluorescence
(Ex.: Rhodamine, DsRed)

3.  Click the button on the Fluoview software



4. Focus to the specimen

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■



Hand switch

*DIC prism



3



Use this knob to adjust the differential interference contrast.

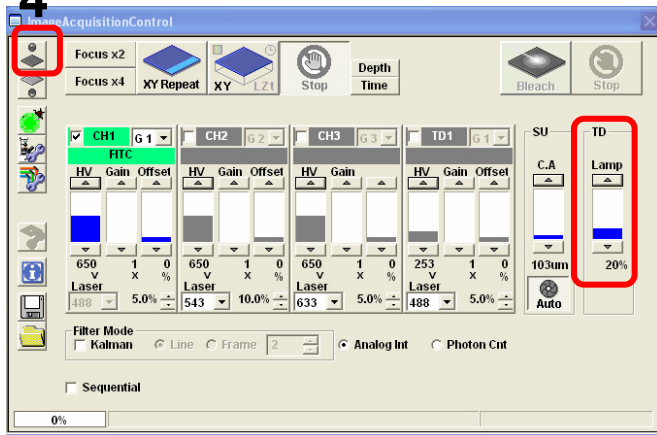
1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software

4



5. Focus to the specimen

Overview of Operation Panel for Image Acquisition

The image displays three screenshots of the image acquisition software interface, with various components labeled and explained.

AcquisitionSetting (Top Screenshot):

- Scan mode**: Points to the Mode section.
- Scan speed**: Points to the Speed section (Fast, 2.0usPixel, Slow).
- Number of pixels**: Points to the Size section (X, Y).
- Zoom & Pan**: Points to the Area section (PanX, PanY, Zoom).
- Laser output adjustment**: Points to the Laser section (458, 488, 515, 543, 633 nm).
- Objective lens**: Points to the Objective section (CHS1, CHS2).
- Focus**: Points to the Focus section (Start, End, StepSize, Resolution).
- Time Interval & Time Number (for acquisition of XYT or XT image)**: Points to the TimeScan section (Interval, Num).

ImageAcquisitionControl (Middle Screenshot):

- Transmitted light observation (visual observation)**: Points to the Focus x2 button.
- Fluorescence observation (visual observation)**: Points to the Focus x4 button.
- DyeApply**: Points to the DyeApply button.
- Optical path diagram**: Points to the Optical path diagram button.
- TwinScanner setting**: Points to the TwinScanner button.
- Save acquisition conditions**: Points to the Save button.
- Load acquisition conditions**: Points to the Load button.
- Scan buttons**: Points to the Scan buttons (XY Repeat, XY, L, Z, T, Stop).
- Select XYZ, XYT or XYL**: Points to the Select XYZ, XYT or XYL button.
- Adjustment of each channel**: Points to the Adjustment of each channel section (CHS1, CHS2, CH3, TD1).
- Confocal aperture**: Points to the Confocal aperture button.
- Light intensity adjustment for halogen bulb**: Points to the Light intensity adjustment for halogen bulb button.
- Kalman**: Points to the Kalman button.

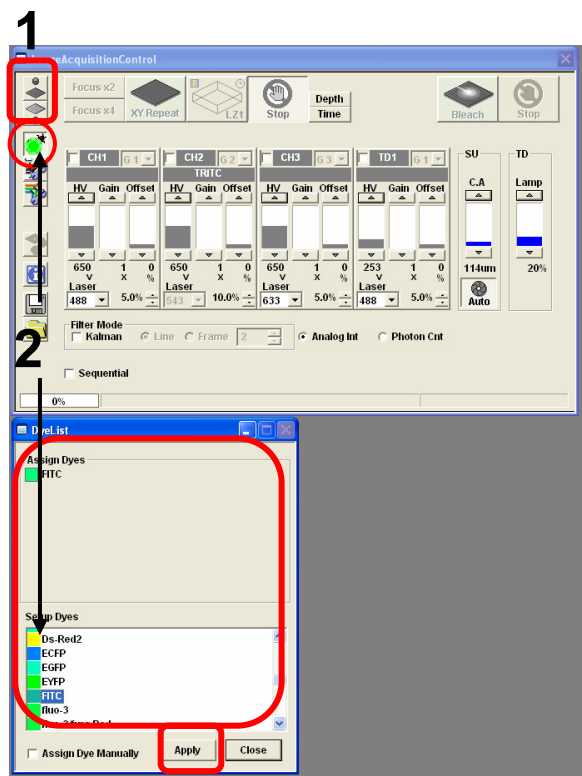
Image View (Bottom Screenshot):



- Image display window**: Points to the Image display window.
- Image file thumbnail**: Points to the Image file thumbnail.
- Display of files in the memory**: Points to the Display of files in the memory section.

Image Acquisition (Single Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Single stain of green fluorescence dye (FITC)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

(The DyeList panel can be closed by using the Close button.)

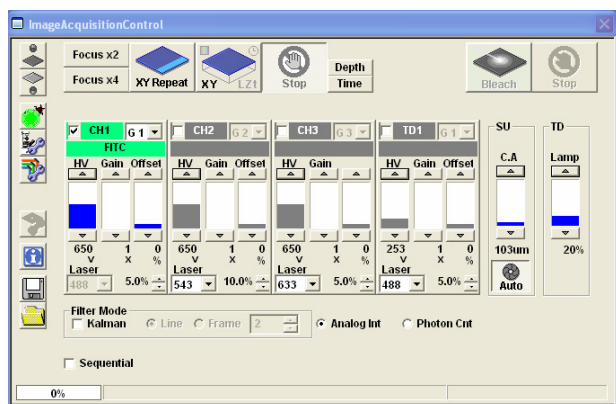
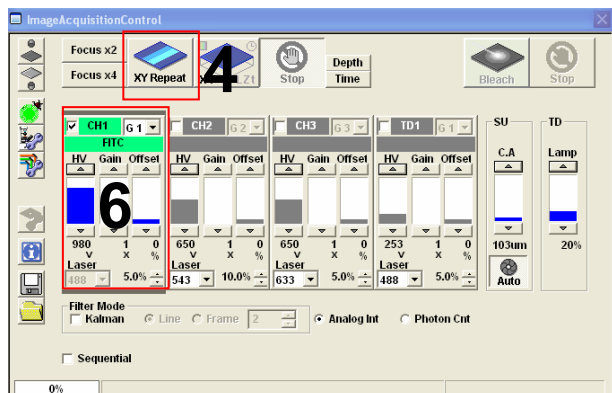


Image Acquisition (Single Stain on XY Image)



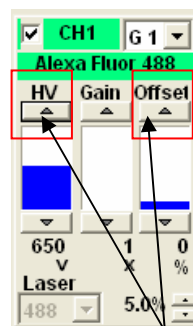
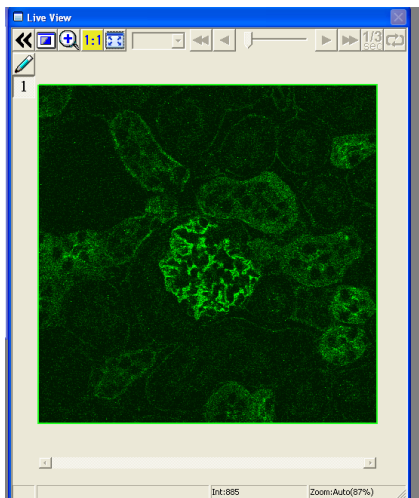
4. Press XY Repeat button click to get image



: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



· Adjust sensitivity of **HV** and reduce noise by **offset**

7. Press keyboard **Ctrl + H key**

Optimized PMT adjustment brightness intensity 2 color between white and black,

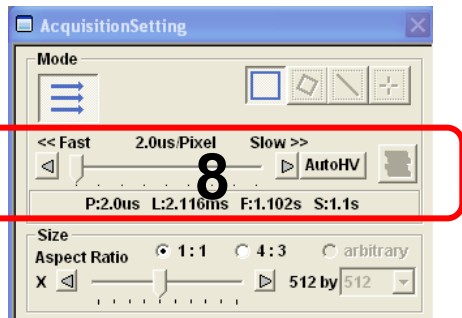
Maximum intensity is 4095 (12bit) if intensity is over 4095, color is changed to red (saturation)

* Basically, **Gain value is 1**

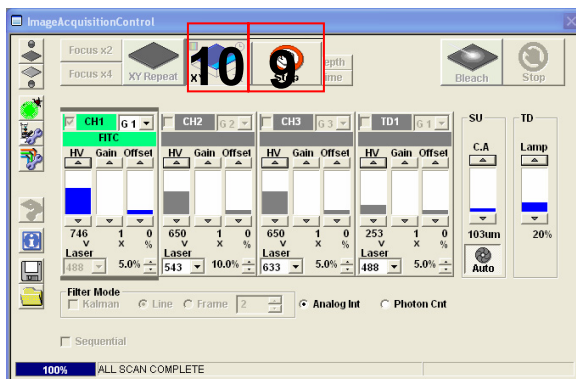



7


Image Acquisition (Single Stain on XY Image)

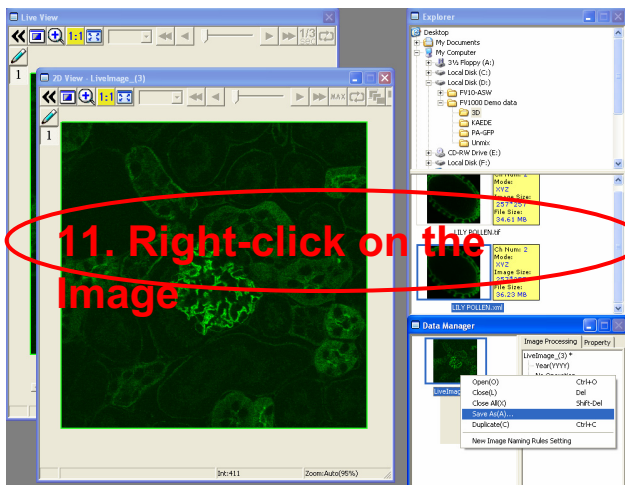


8. Select AutoHV and then select ScanSpeed.
*As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



9.  Press the Stop button to stop scanning.

10.  Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



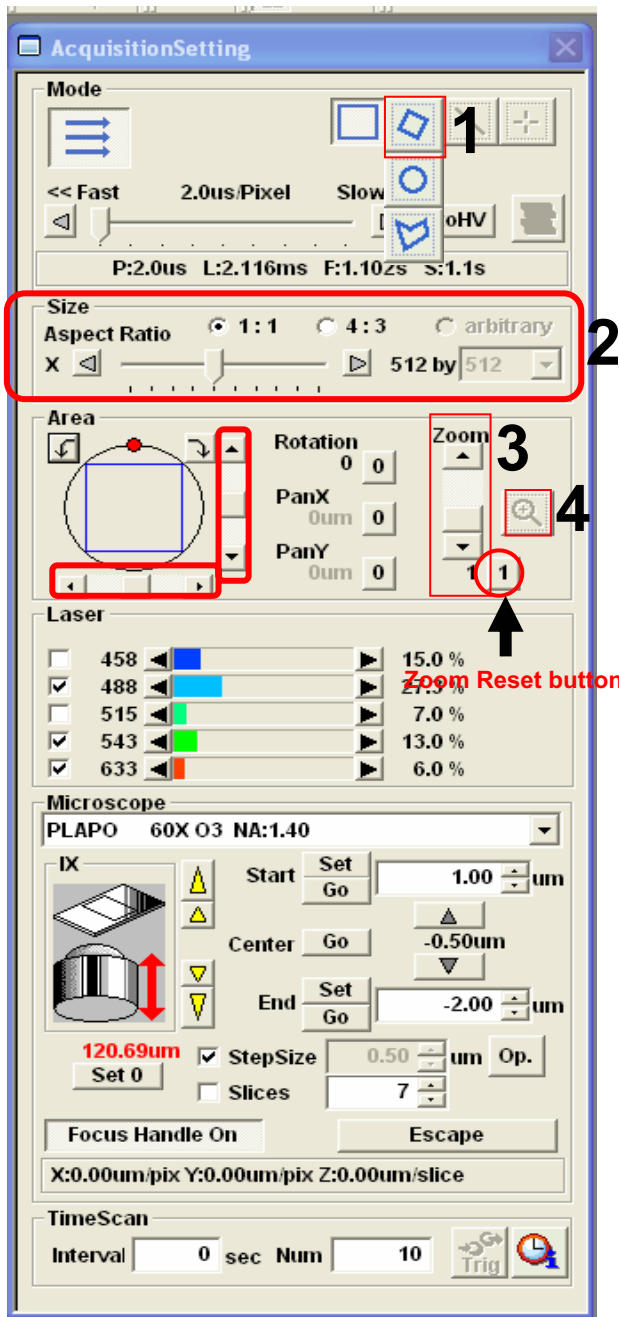
11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

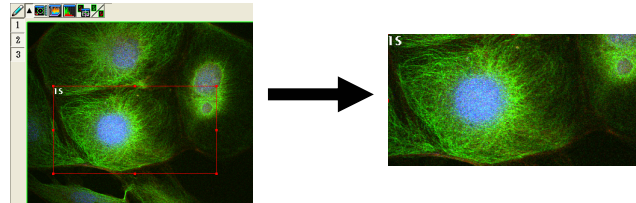
Save the image as TIFF, BMP, JPEG format Select "Export " and chose the format TIFF, BMP, JPEG.

■Memo■
File formats specifically for the FV10-ASW
OIF format:
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.
OIB format:
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Complement of adjusting the image



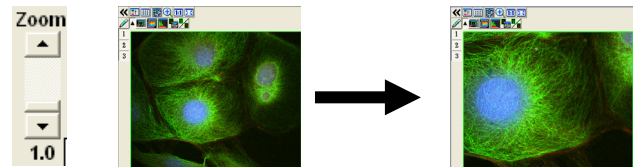
1. Click "Clip scan" button , and enclose an interesting region's image on the whole image.




2. pixel setting
* The standard pixel is 512 x 512

3. Zoom Setting

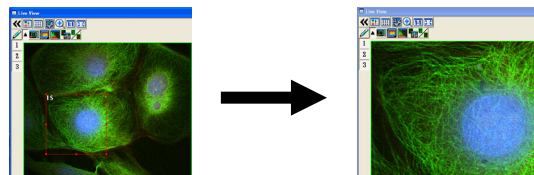
Press "XY Repeat" to scan and set zoom value.



Above image is zoomed From 1 x to 2
* Scan speed and pixel resolution remain even zoom value is changed

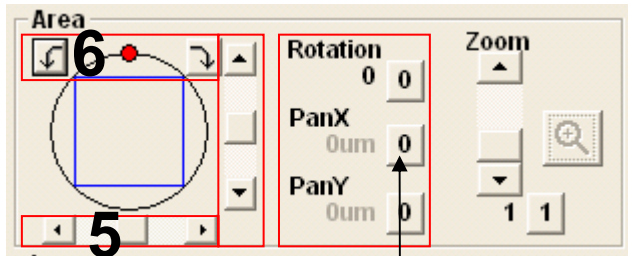
4. Click  Zoom scan, and be able to enclose an interesting region's on the whole image

Press XYRepeat to scan after enclosing the region

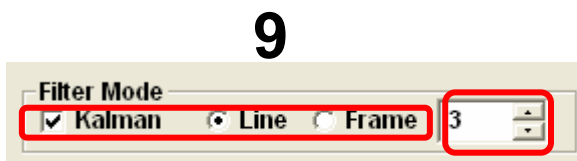
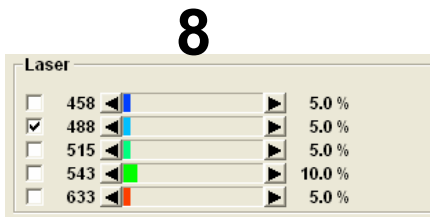


* Scan speed and pixel resolution remain even zoom value is changed

Complement of adjusting the image

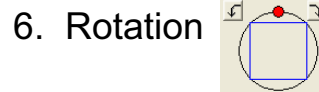


PanX,Y and Rotation reset button




5. Pan X,Y

Be able to move the field of view to set Pan X,Y without stage action



6. Rotation

Be able to rotate the whole image.

7.  Click "Auto" button to acquire Optimized Confocal aperture ... change confocal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity ... More Laser intensity is increase , more bright image is .

* More increase laser intensity is , more discoloration image is .

9. Kalman accumulation ... Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

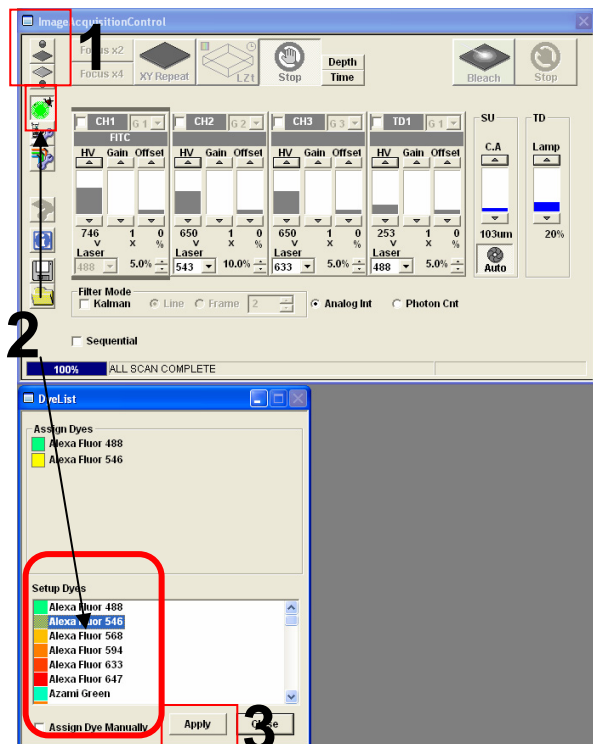
Disadvantage: Some blur occurs due to averaging of images.



Image Acquisition (Double Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



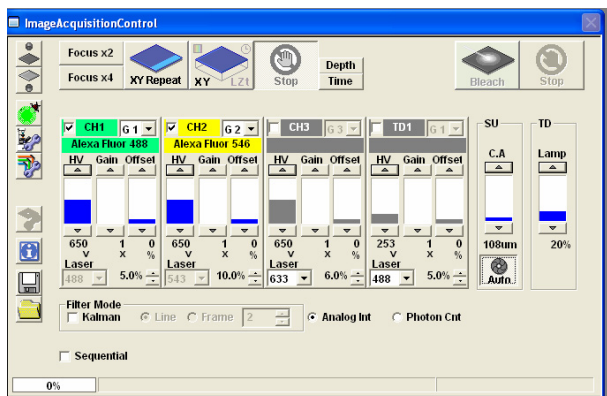
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

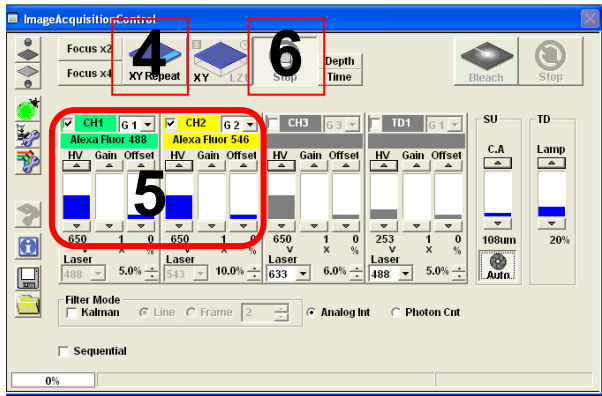
3. Click “Apply” button.

(The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

Image Acquisition (Double Stain on XY Image)



4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

(The image adjustment is outlined below.
For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■

Scan buttons



: Continuous scan

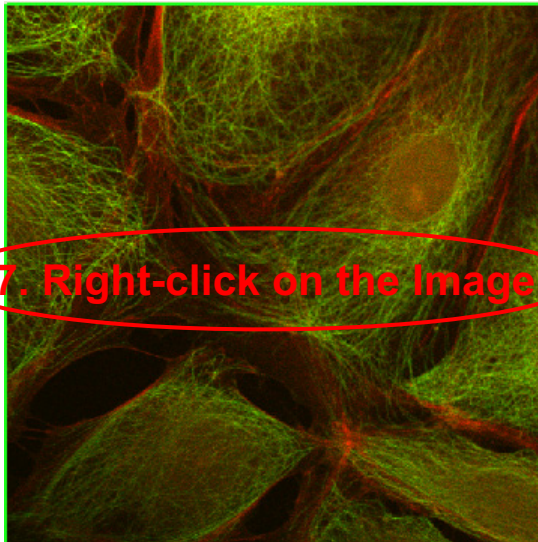


: Stop scan



: Rough scan (Line skipped)

7. Right-click on the Image



7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

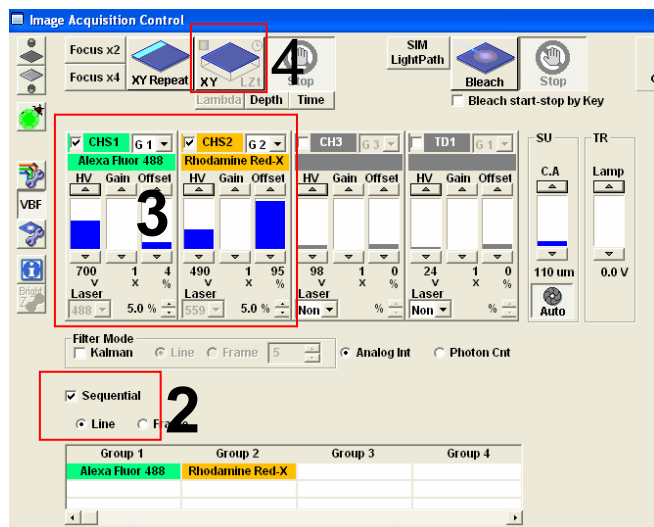
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

Image Acquisition (Double Stain on XY Image)

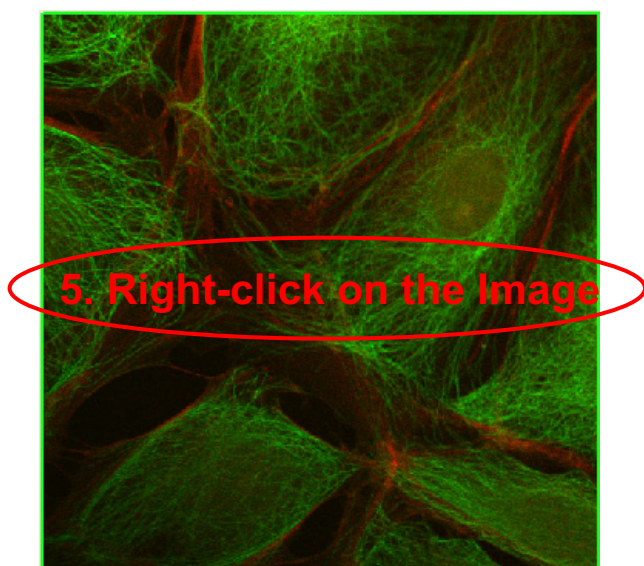
■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)



1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.
2. Check Sequential and select Line.
3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
4. Press the XY button to acquire an image.
5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.) The image is acquired.



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

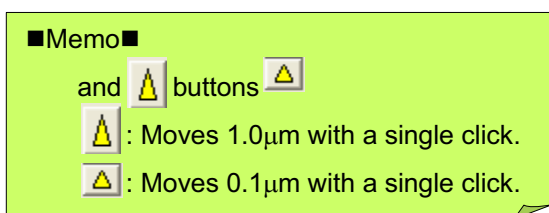
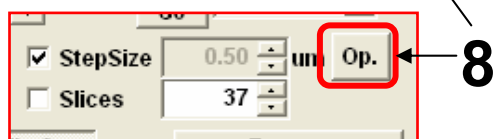
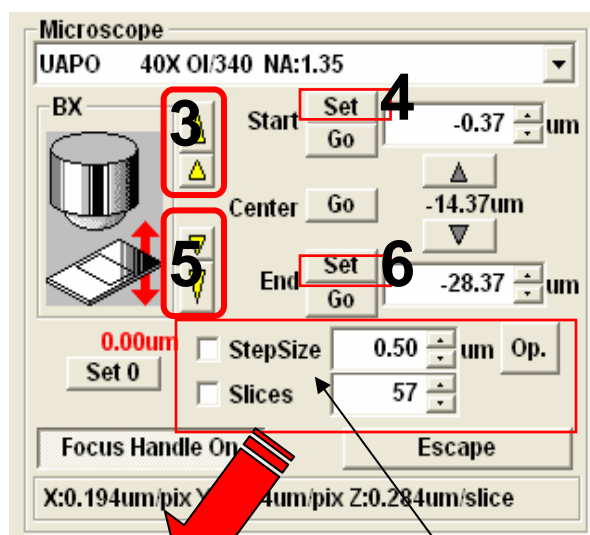
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Double Stain on XYZ Image)

■ Acquisition of 3D images (XYZ) (fluorescence image only) ■

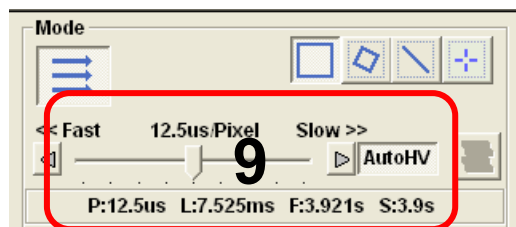
Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



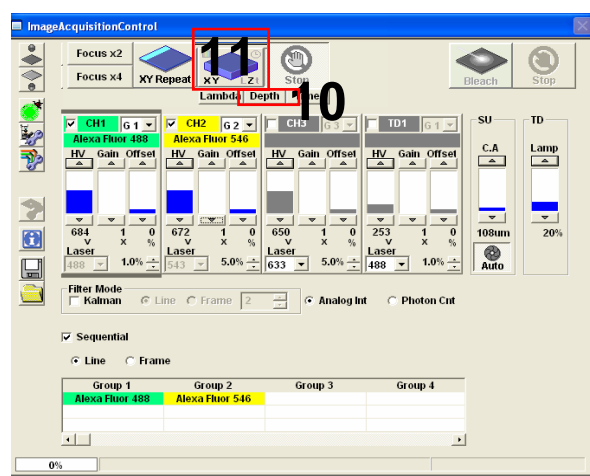
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

Image Acquisition (Double Stain on XYZ Image)



9. Select AutoHV and then select ScanSpeed.

10. Select Depth.



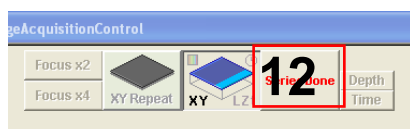
11. Press the XYZ button to acquire an image.

12. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:

Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

13. Right-click on the Image

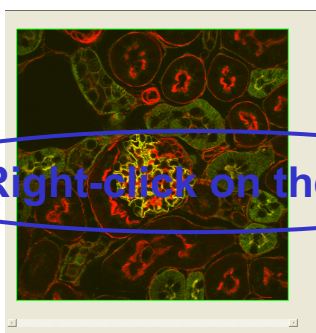
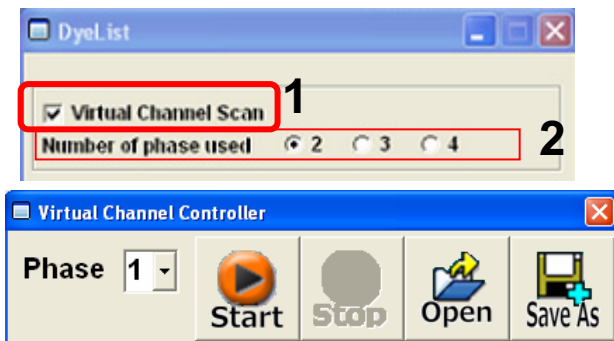


Image Acquisition (Four Stain on XY Image)

■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■

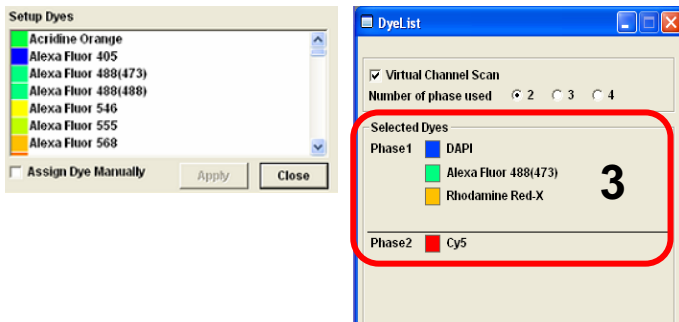
Sample: Four stain of Blue fluorescence dye (DAPI) ,green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

This is the procedure to acquire images through Virtual Channel scan

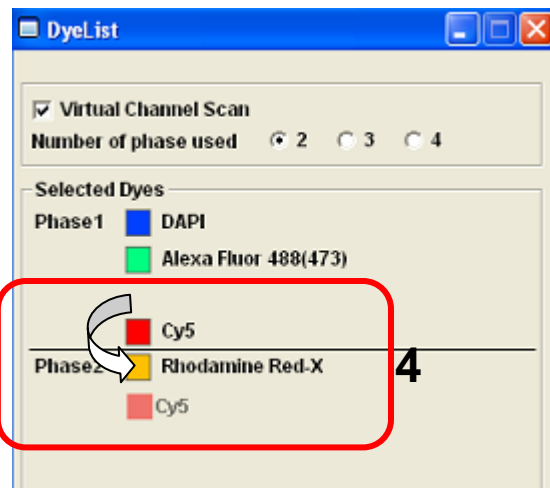


1. ☒ Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then **“Virtual Channel Controller”** is automatically turned on.

2. Select a number of Virtual Channel from **“Number of phase used”**.



3. Select 4dyes from DyeList 4th dye is registered in **“the Phase 2”**.



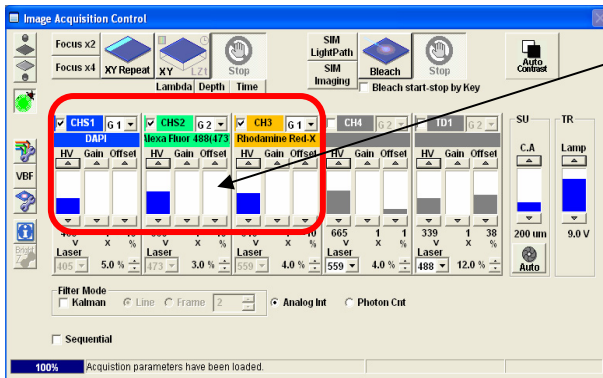
* RodaminRed is able to be registered on **“Phase2”** to drag .

Image Acquisition (Four Stain on XY Image)



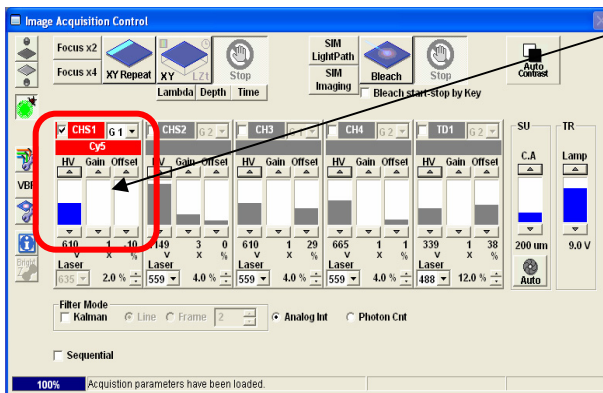
4. Select "Phase1", "DAPI", Alexa488, RhodaminRed are registered on ImageAquisitionControl.

* Slit and Filter, DM are automatically set for "DAPI", "Alexa488" or "PhodaminRed"

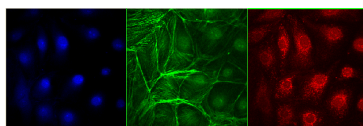


5. Select "Phase2", Cy5 is registered on ImageAquisitionControl

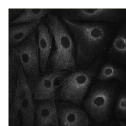
* Slit and Filter, DM are automatically set for "Cy5"




Adjust the image at each phases
"Phase1" "Phase2"



6




6. Adjust the image to click  "XY Repeat" at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

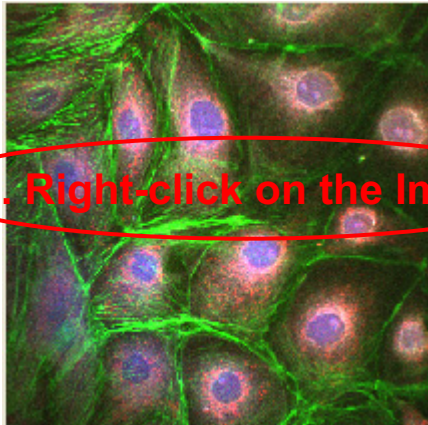
Image Acquisition (Four Stain on XY Image)



7

7. Click  on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.

■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

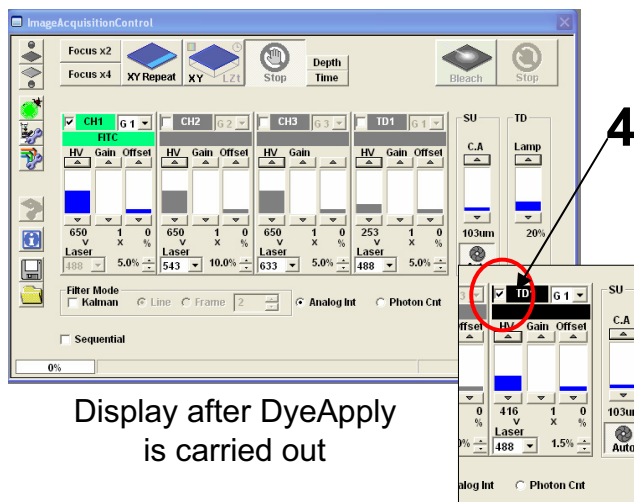
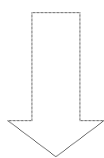
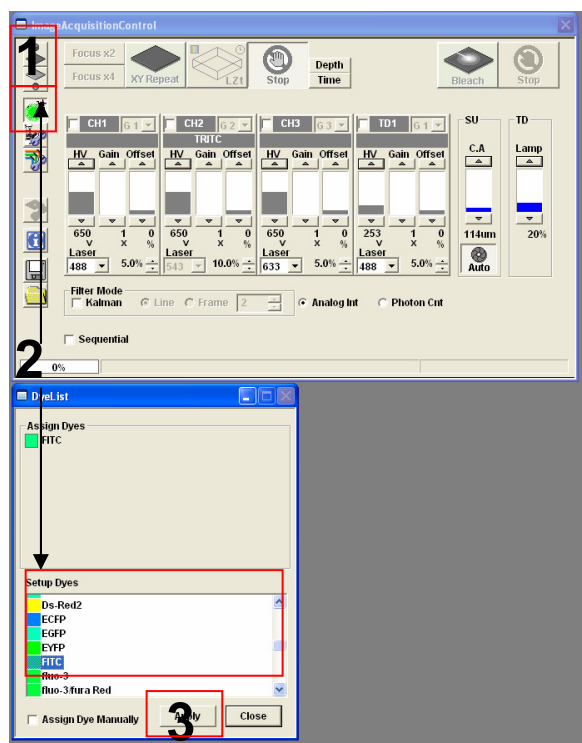
OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.



Image Acquisition (Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image



Display after DyeApply
is carried out

1. Click on the FV10-ASW software
button  to close the fluorescence
lamp shutter.
Alternatively, click on the  button
to close the halogen bulb shutter.

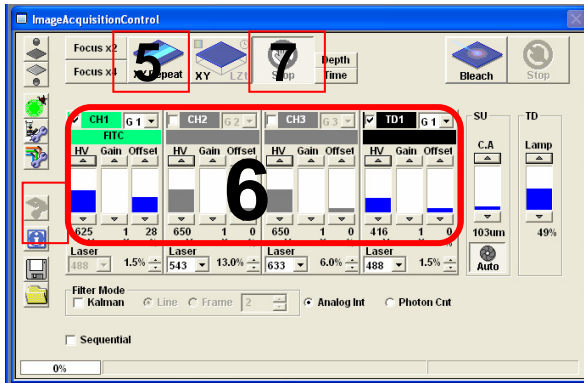
2. Click on the DyeList button. On the
DyeList panel, double-click on a
fluorescence reagent to be used for
observation.

* To cancel the selection and select a
different reagent, double-click on the
fluorescence dye listed on the Assign
Dyes window and take step 2 again.

3. Click on the Apply button.
(The DyeList panel can be closed by
using the Close button.)

4. Check TD1.

Image Acquisition (Single Stain + DIC on XY Image)



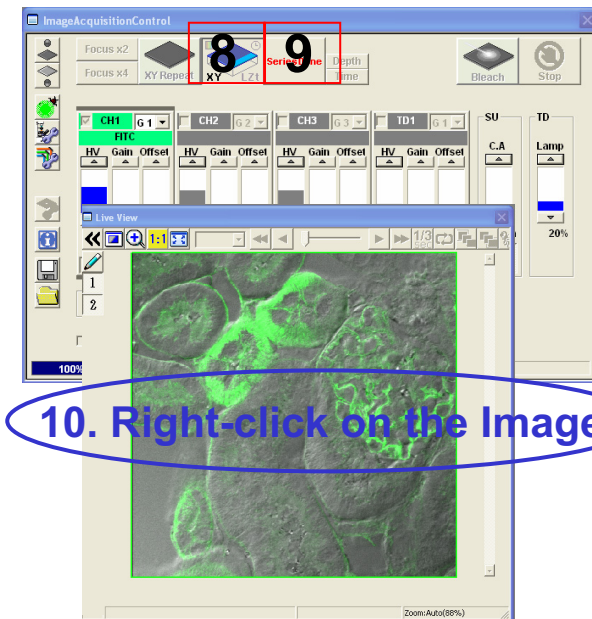
5. Press the “XY Repeat” button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the “Stop button” to stop scanning.

8. Press the “XY button” to acquire an image.

9. Click on “SeriesDone”, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.



10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

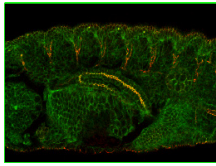
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

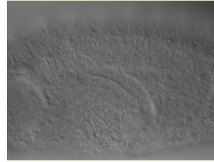
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Merge the images between fluorescent XY image and DIC image

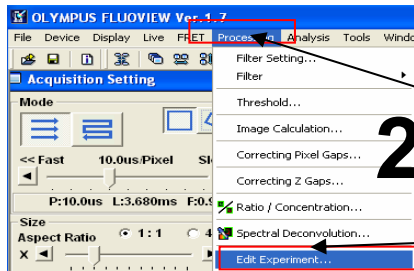
Edit different each files to the same file. This is available for making merge image
Between fluorescent image and focused DIC image.



1

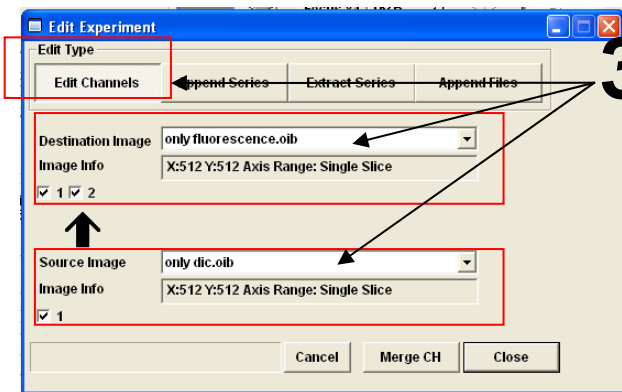


1. Open fluorescent image and
DIC image.



2

2. Select
Edit experiment from **Processing**

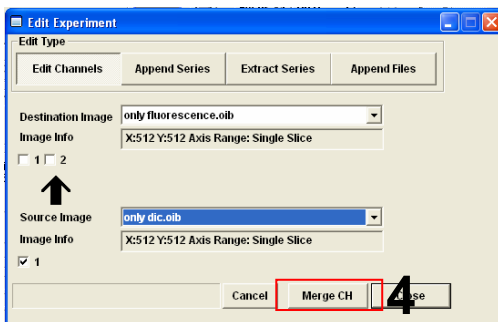


3

3. Click **Edit Channels**, and select
fluorescent image file at
Destination Image, select DIC
image at **Source Image**.

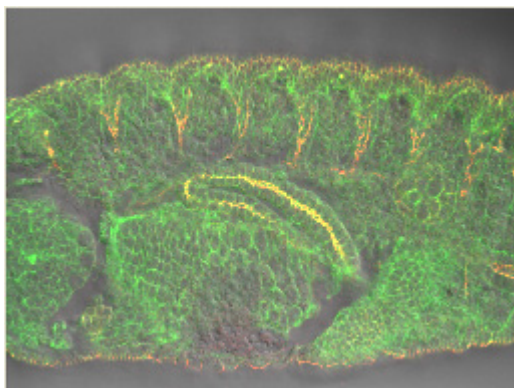
Image Info

* Check Image Info ☒ 1 ☒ 2 to make the.
merge file. If all channels are checked, all
channels are reflected in the new merged file.



4

4. Click **Merge CH**, and then
the fluorescent image and the DIC
image are merged as the new file.

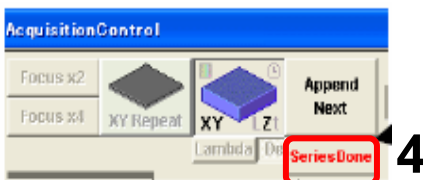
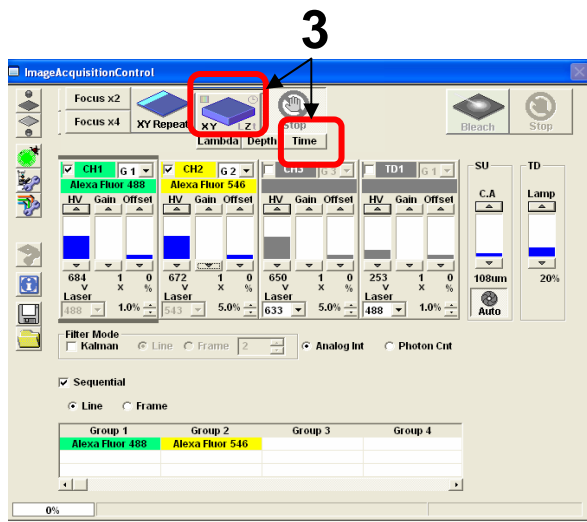
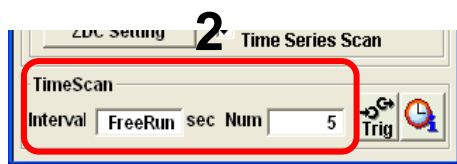


5

5. Merged image between the
fluorescent image and the DIC
image.

Image Acquisition (Single Stain on XYZT Image)

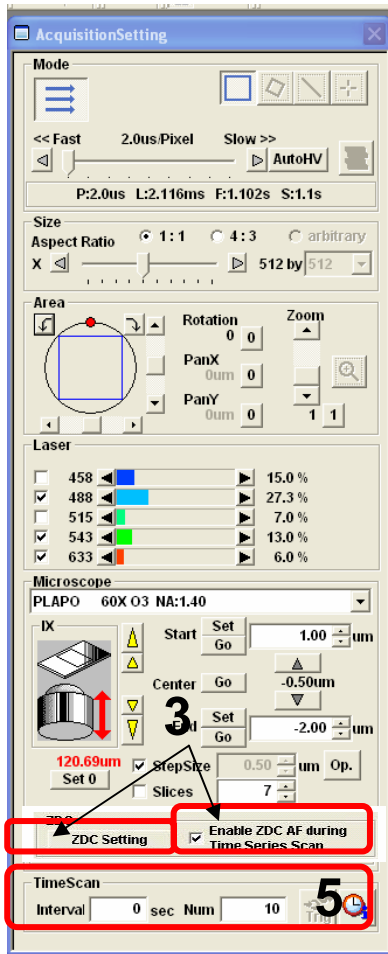
This is available for the Time series scan experiment.



1. Adjust the image.
* Refer P17,18
2. Enter interval time to “Interval”
Enter interval number to “Num”

Example: Acquiring time series scan images every 5minutes for 1hour is below,
3. Select “Time” and then click XYTbutton to acquire Time series scan image.
4. Click on “SeriesDone”, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.

Image Acquisition (Single Stain on XYZT Image)



1. Adjust the image.

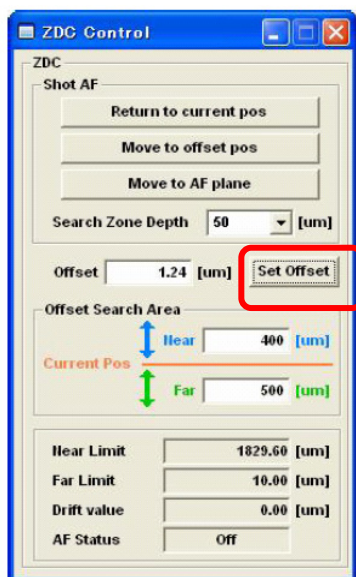
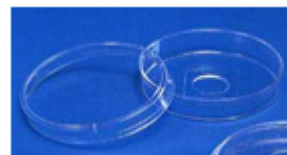
* Refer P17,18

2. Insert ZDC unit to left side.

3. Check “**EnableZDC AF during Time Series Scan**” and click “**ZDC setting**” .

4. Click “**Set Offset**” to register auto focus position.

* **Note: Have to use glass bottom dish below, otherwise ZDC doesn't work.**



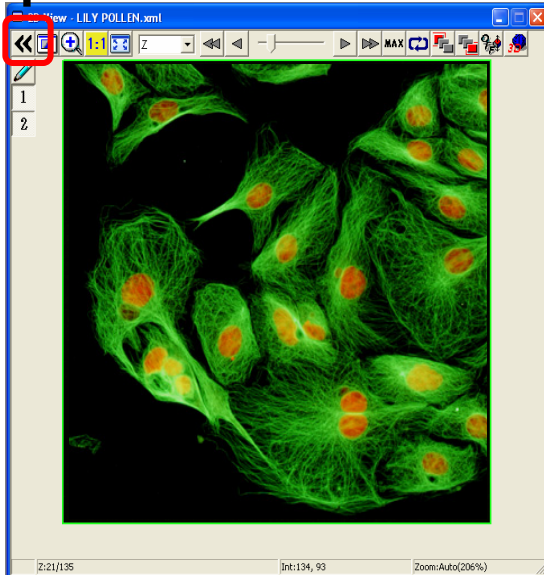
5. Set “**Interval**” and “**Num**” and then click “**XYZT**” to acquire the time series image.

* **Note: In case of using ZDC for Time series Scan, follow below limits**
Interval number is more than 60 sec,
Rest Time is more than 30 sec,
otherwise ZDC doesn't work.

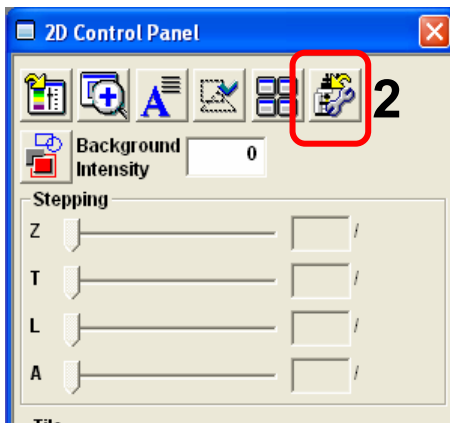
* If use “TimeControler”, Time Series Scan is able to done even interval number is within 60sec and Rest Time is within 30sec.

Reload the image conditions

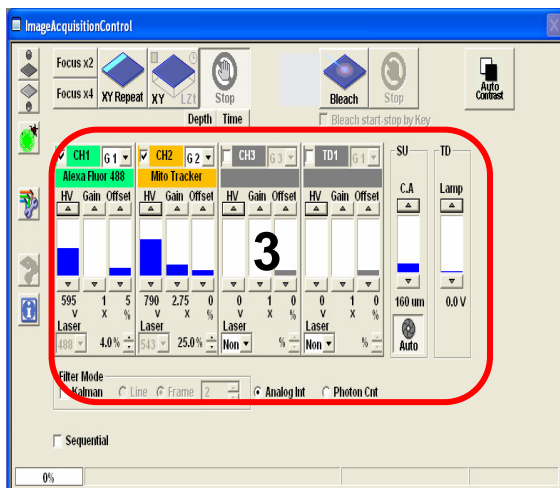
1



1. Open the file and click



2. Click



3. The conditions (HV,Offset, CA and so on) are reloaded .

Overview of the 2D Operation Panel

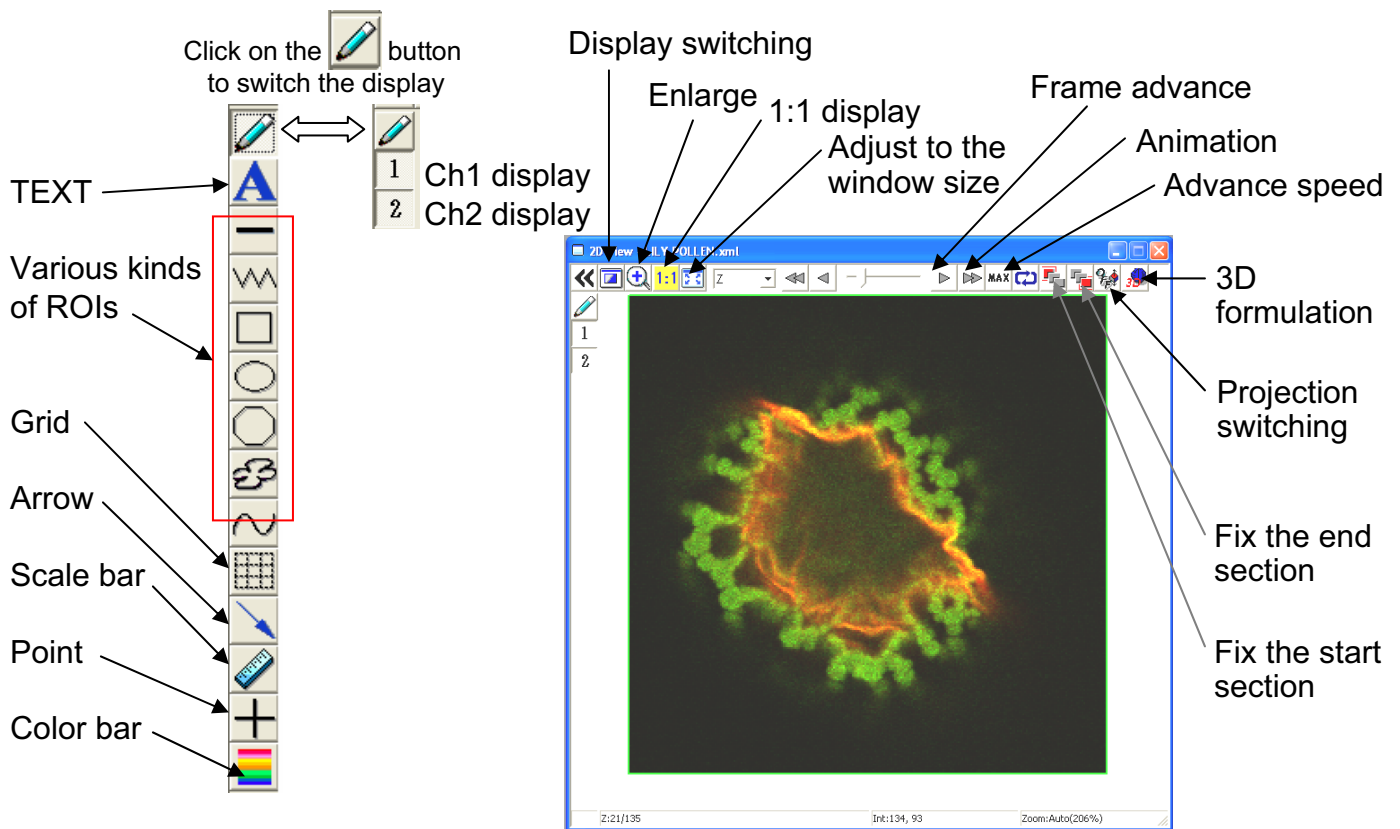
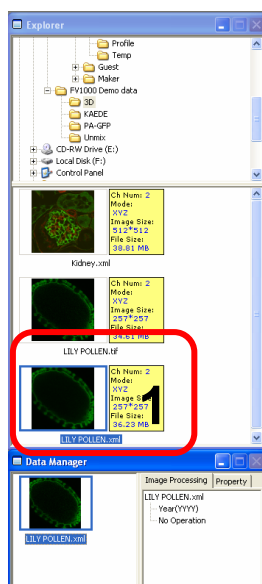
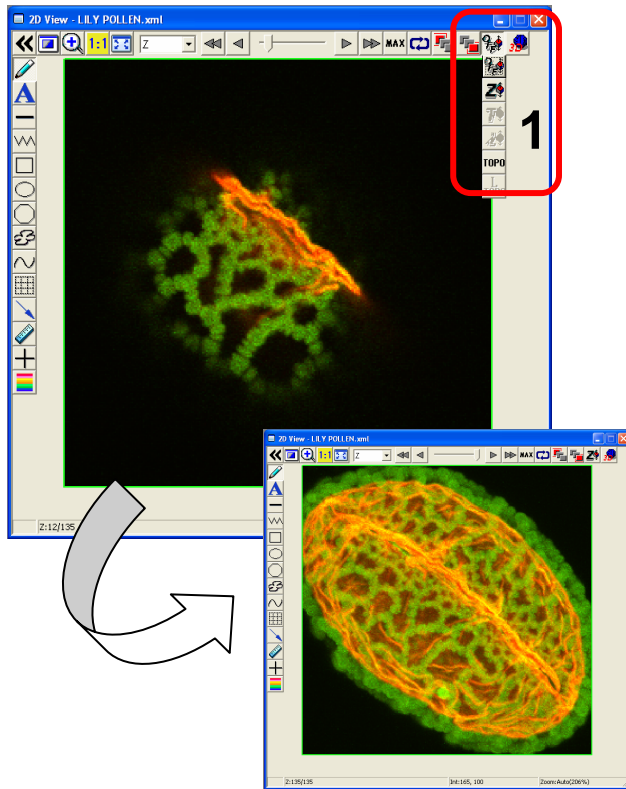


Image Analysis (Opening a File)

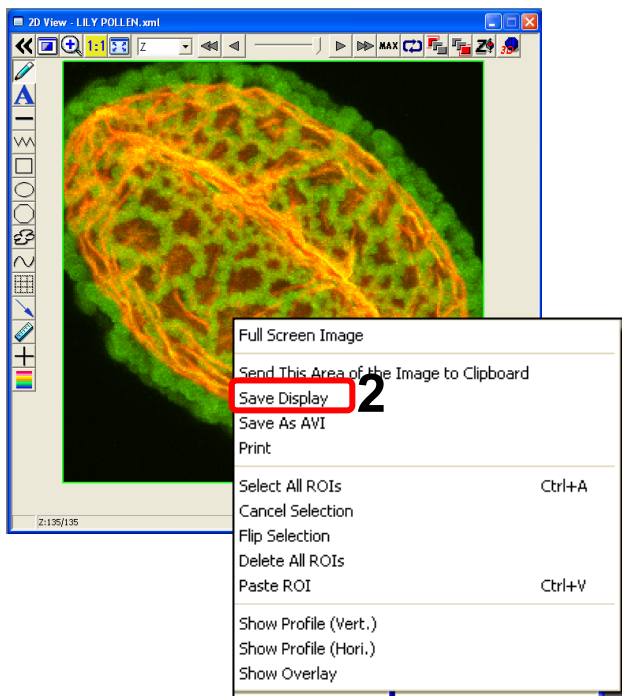


1. Double-click on a file to be opened from Explorer.

Image Analysis (Acquire a Projection Images)



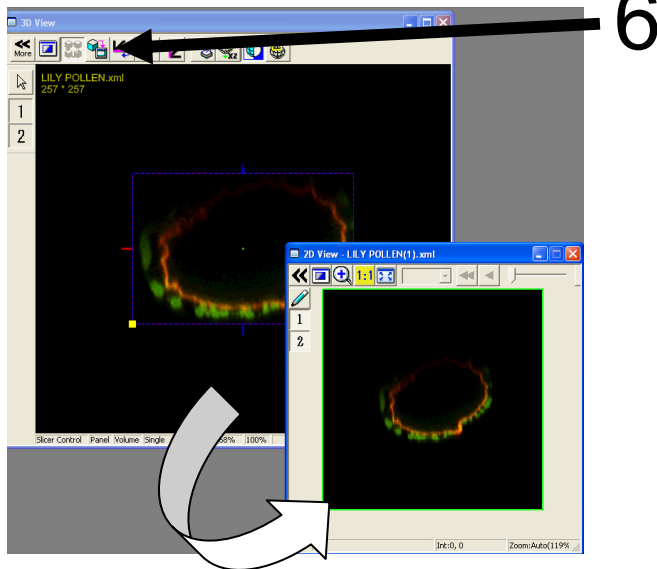
1. Click on the  button to select .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

Image Analysis

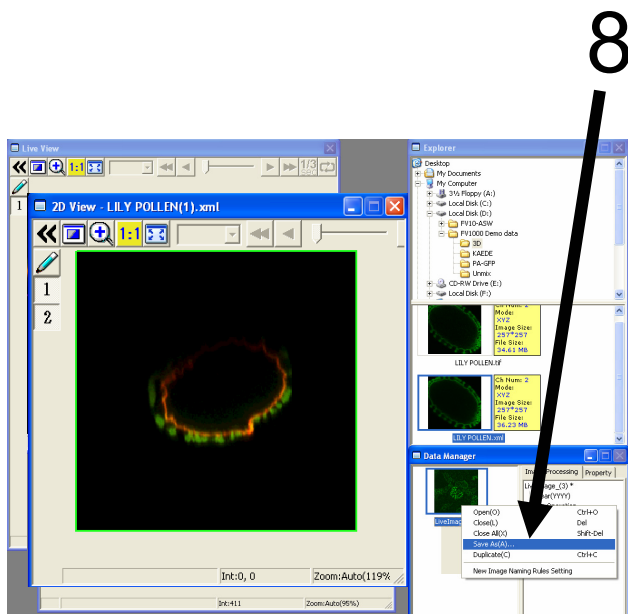
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

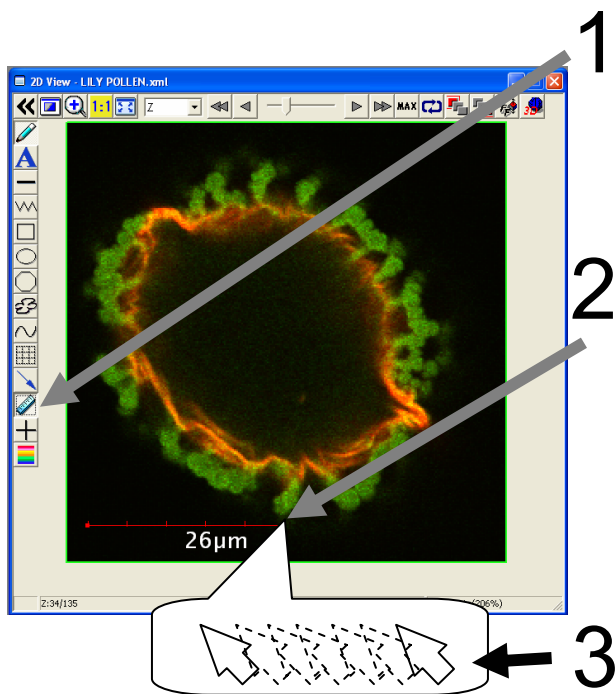
OIF format:


Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Analysis (Inserting the Scale Bar)

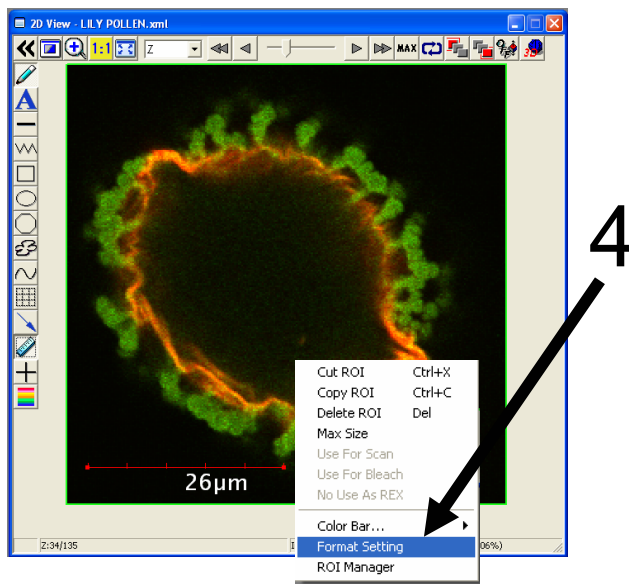


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

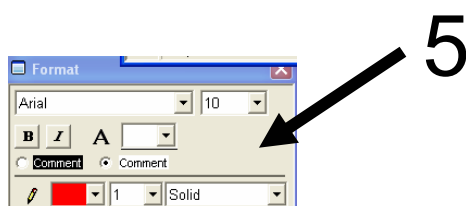
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

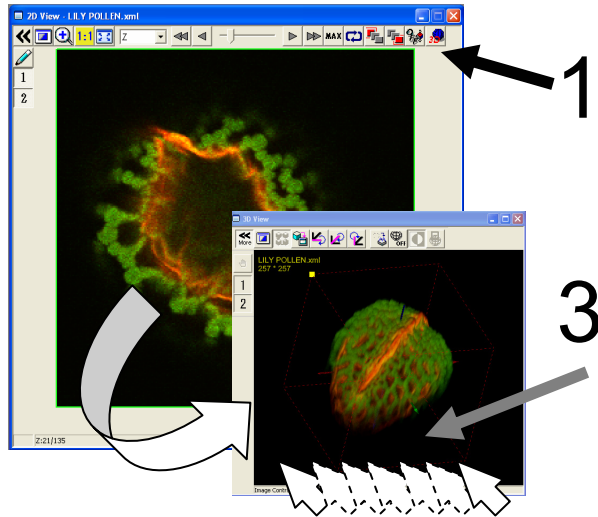
4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.




5. Change the setting in this window as required.

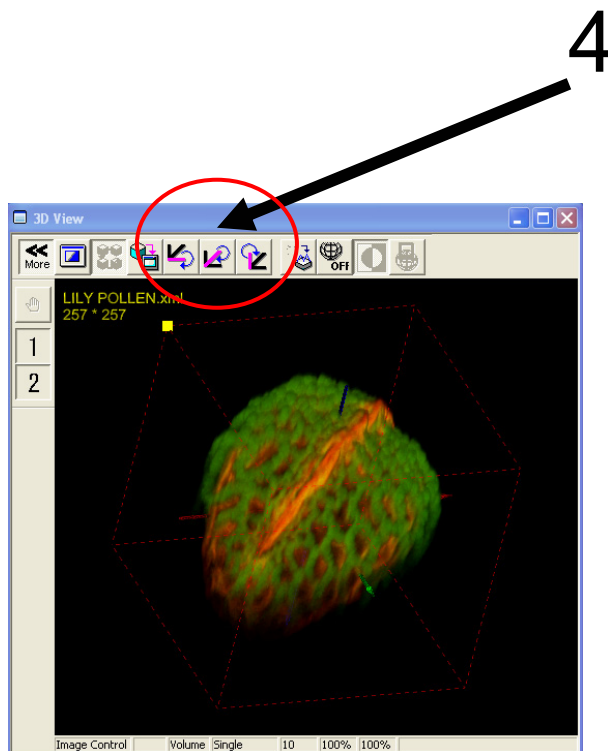
Image Analysis


(Rotating a Three-dimensional Image)




1. Click on the  button for a 2D View-(file name) image.
2. A 3D view is created.
3. Drag the mouse on the image to observe it at a certain angle.

Simple animation



4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.


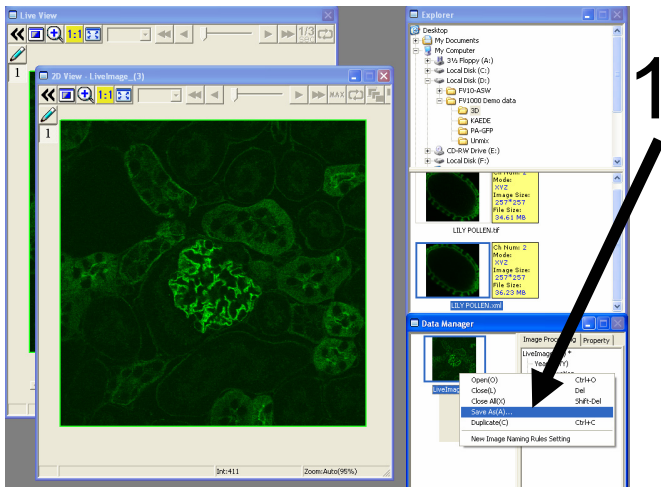
Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

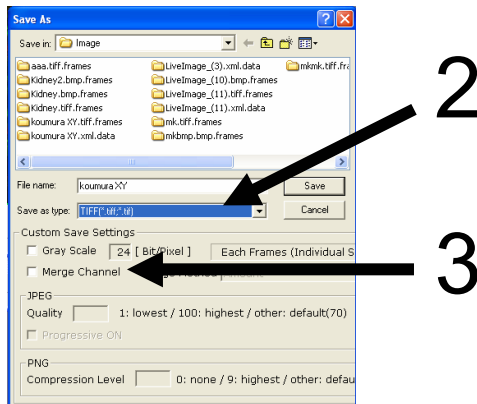
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

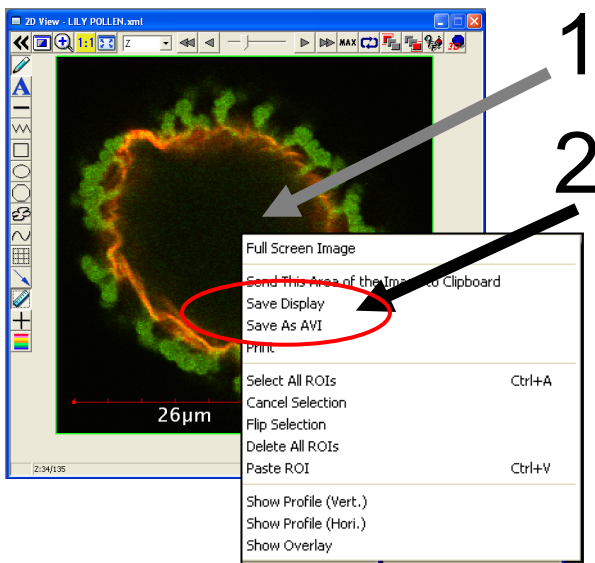
* BMP and JPEG formats are also selectable.



Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.



Convert an image with the scale bar inserted into a BMP format

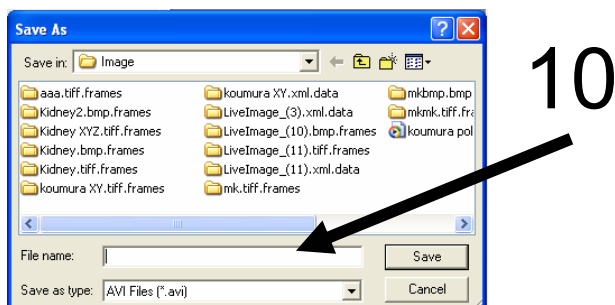
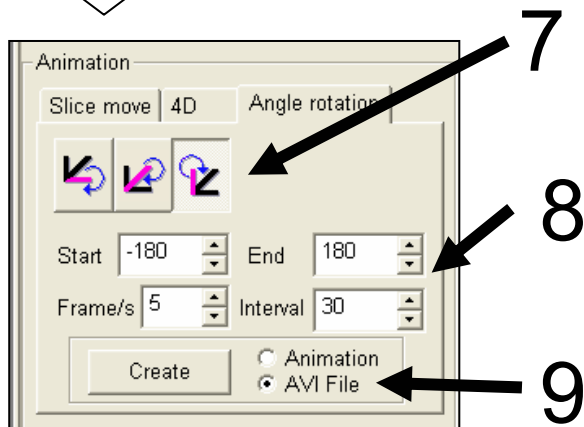
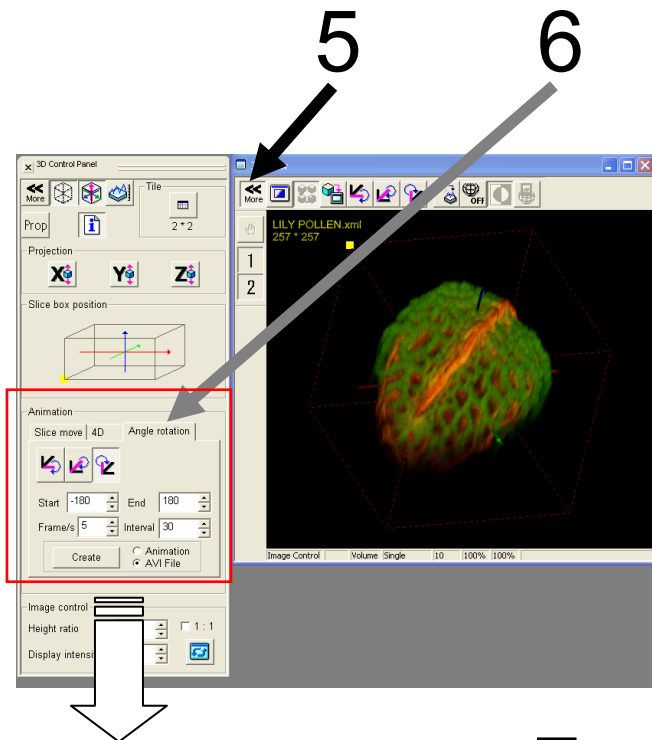
1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

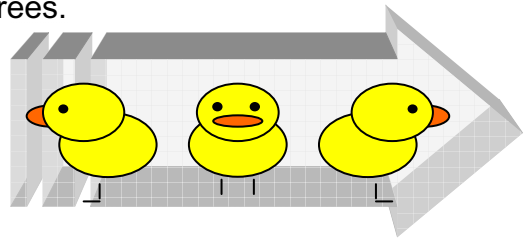
Image Analysis

(Rotating a Three-dimensional animation)



To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the  button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

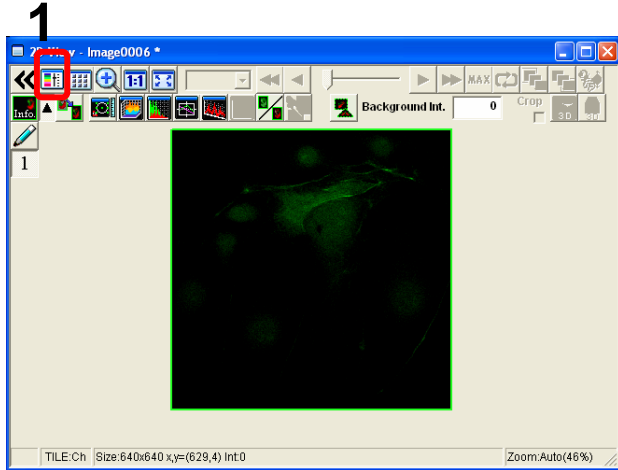
8. Enter the rotation angle.

Start = Angle to start rotation
End = Angle to stop rotation
Frame/s = Rotation speed
Interval = Degrees to be rotated at a time

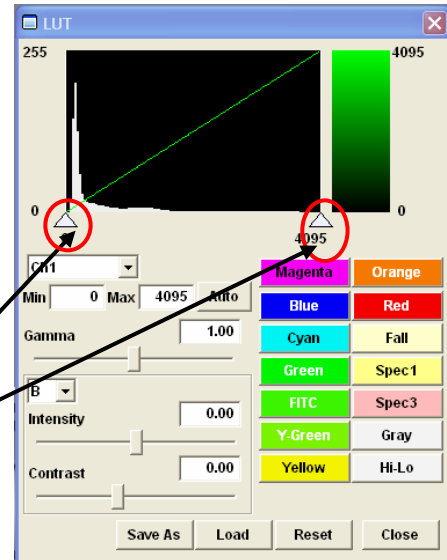
9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

2D Image Analysis (Edit the image color and contrast)




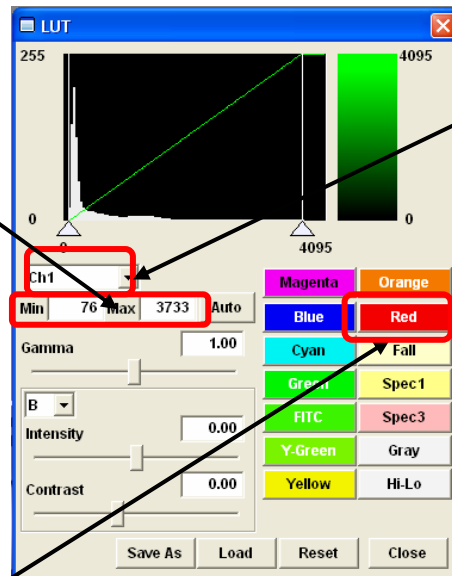
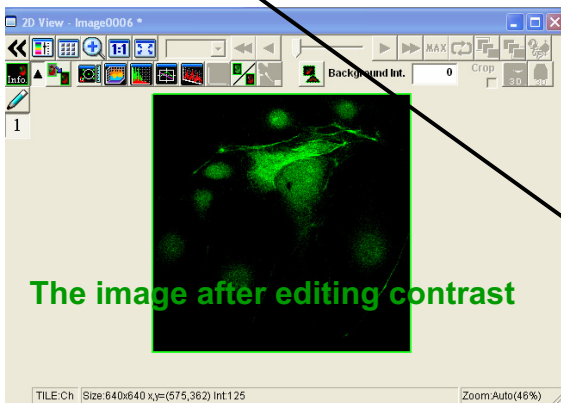
1. Click  "LUT" and then LUT table appears below,



3. Min and Max value are changed and contrast of image is edited.

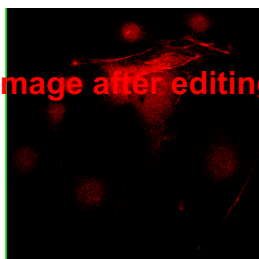
* According to get Min value up , be able to reduce noise of the image.

2. Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min"(Max4095, Min0)



Edit each Ch

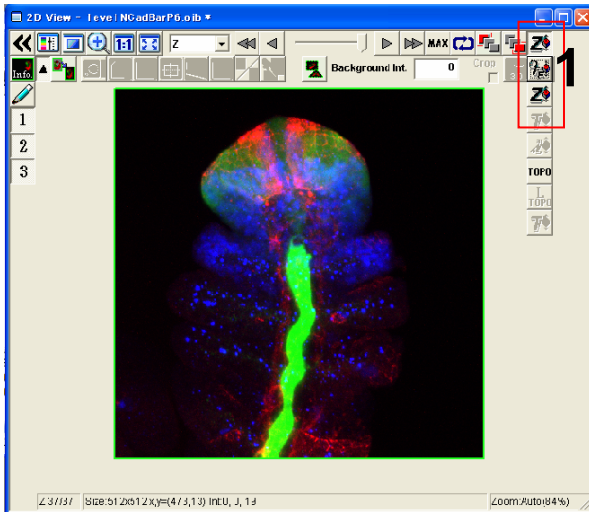
The image after editing color





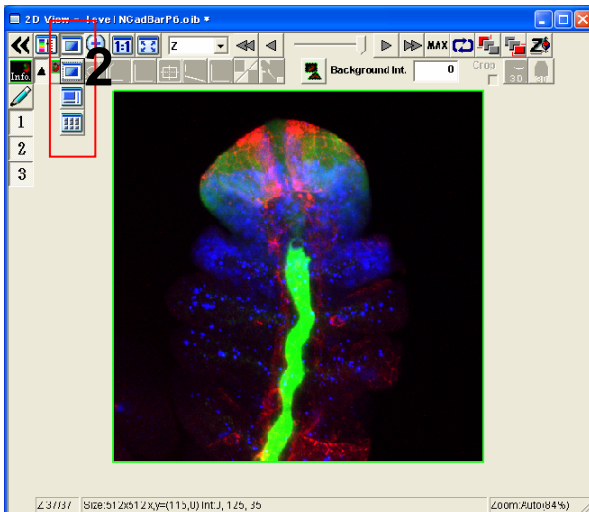
4. To click another color, be able to Edit a color.
Above example: Change Green to Red to click



Red

2D Image Analysis (the image of Z section)

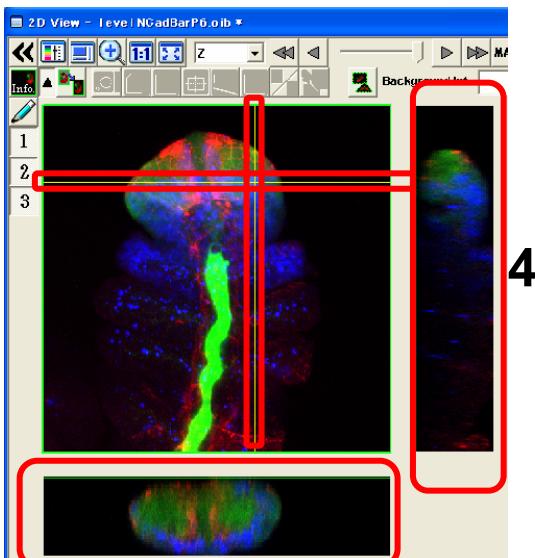


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select .

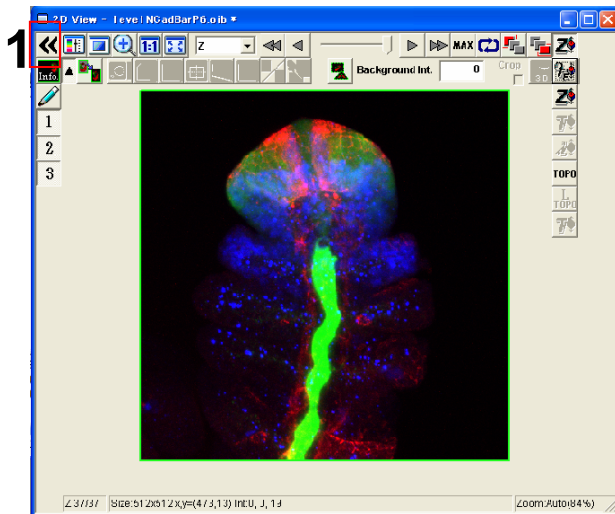
3. The images of Z section is shown on X axis and Y axis.
According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.




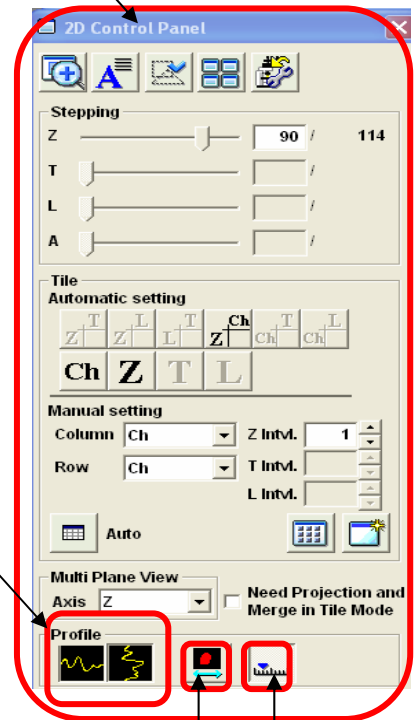
4. The image of Z section on Y axis.

5. The image of Z section on X axis.

2D Image Analysis (Intensity Profile of each Z sections)

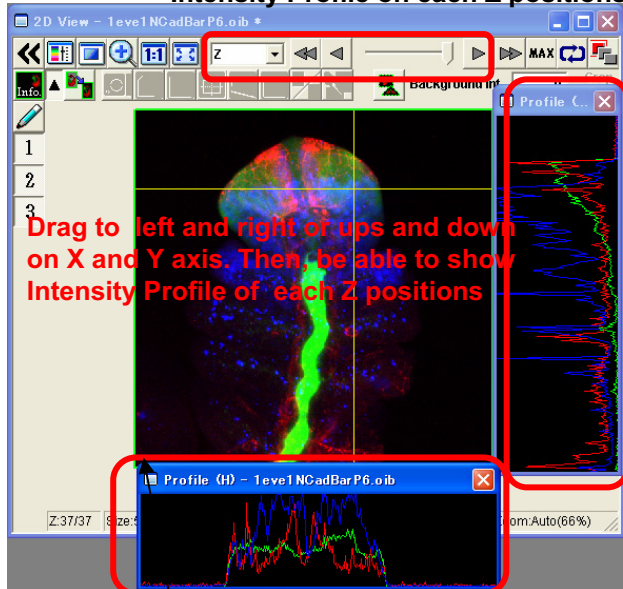


1. Click  and then 2D Control Panel is shown below,




2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

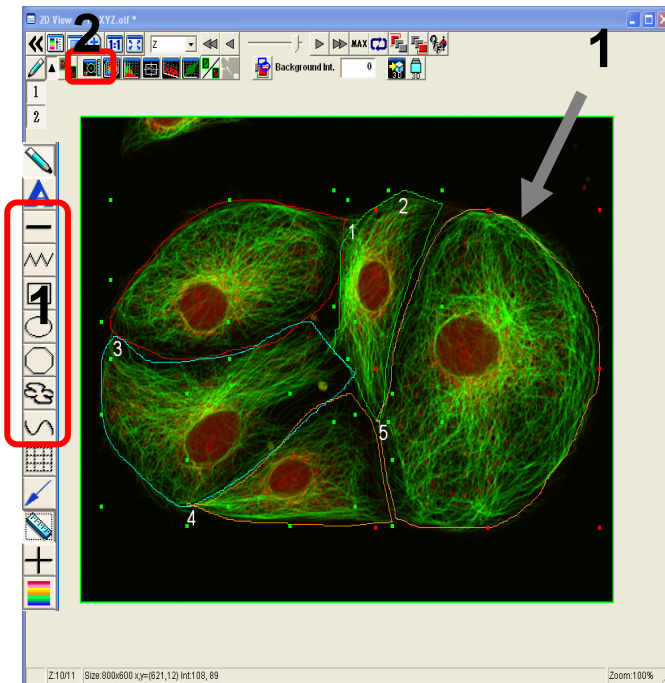
To move to Z position, be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click , be able to show as equal scale of Profile window as 2Dimage.

2D Image Analysis (Measure)



1. Enclose interesting regions by ROI

Line on interesting positions by ROI

2. Click "measure".

4. According to click "Measure All ROIs", then the information of all ROI is calculated on Region Measurement.

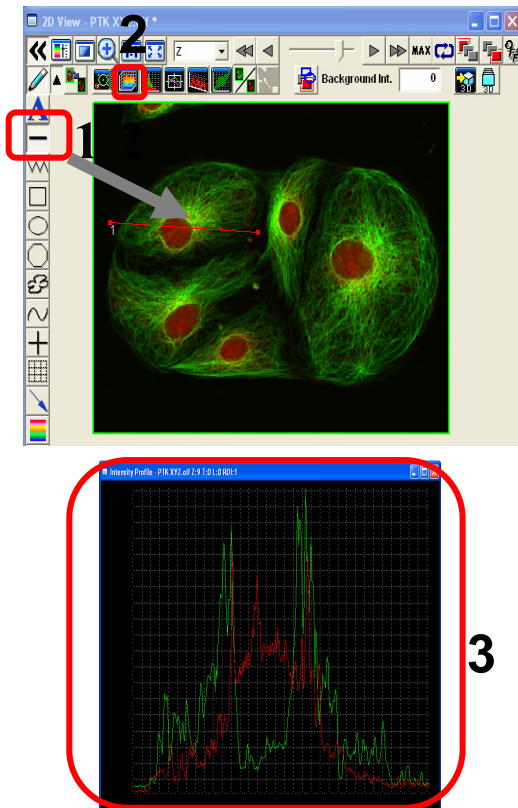
3. The information of ROI is calculated on Region Measurement.

5. The information of all ROIs


ROI	CenterX [um]	CenterY [um]	Area [um*2]	Perimeter [um]	Integration CHS1	Average CHS1	Max CHS1	Min CHS1	Range CHS1	StdDev CHS1	3StdDev CHS1	Integration CHS2	Average CHS2	Max CHS2	Min CHS2	Range CHS2	StdDev CHS2	3StdDev CHS2
1	57.171	48.438	3129.625	241.490	5478264.000	1107.926	4095.000	95.000	4000.000	710.261	2130.783	2952481.000	658.076	3590.000	28.000	3562.000	522.518	1567.554
2	112.522	53.402	1470.188	194.764	9620457.000	1301.724	4095.000	97.000	3998.000	883.602	2650.807	7837013.000	758.280	3468.000	28.000	3440.000	561.877	1685.630
3	51.900	87.103	3274.688	273.215	2573667.000	1003.410	4095.000	94.000	4001.000	700.397	2101.192	9839166.000	569.504	3415.000	53.000	3362.000	443.623	1330.869
4	80.180	111.524	1732.438	211.246	4386227.000	879.766	3836.000	83.000	3753.000	657.656	1972.967	7880740.000	645.072	3380.000	25.000	3355.000	523.061	1569.183
5	150.780	79.732	6120.813	313.258	1878548.000	1244.509	4095.000	96.000	3999.000	725.103	2175.309	4771708.000	559.277	3227.000	41.000	3186.000	439.334	1318.002

Count	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Average	90.511	76.240	3145.550	246.795	5987432.600	1107.467	4043.200	93.000	3950.200	735.404	2286.212	9656221.600	638.042	3416.000	35.000	3381.000	498.083	1494.1
Max	150.780	111.524	6120.813	313.258	1878548.000	1301.724	4095.000	97.000	4001.000	883.602	2650.807	4771708.000	758.280	3590.000	53.000	3562.000	561.877	1685.6
Min	51.900	48.438	1470.188	194.764	4386227.000	879.766	3836.000	83.000	3753.000	657.656	1972.967	7837013.000	559.277	3227.000	25.000	3186.000	439.334	1318.0
Range	98.879	62.087	4650.625	118.495	7492321.000	421.958	259.000	14.000	248.000	225.947	677.840	5934695.000	199.002	363.000	28.000	376.000	122.543	367.0
StdDev	41.309	25.569	1848.840	47.735	8699715.061	172.621	115.828	5.701	110.244	86.561	259.683	5124831.492	80.326	132.286	11.811	137.208	54.102	162.3
3StdDev	123.928	76.707	5546.521	143.205	8699145.184	517.864	347.485	17.103	330.731	259.683	779.050	5374494.476	240.979	396.857	35.433	411.624	162.305	486.9

2D Image Analysis (Line Intensity Profile on the 2D image)



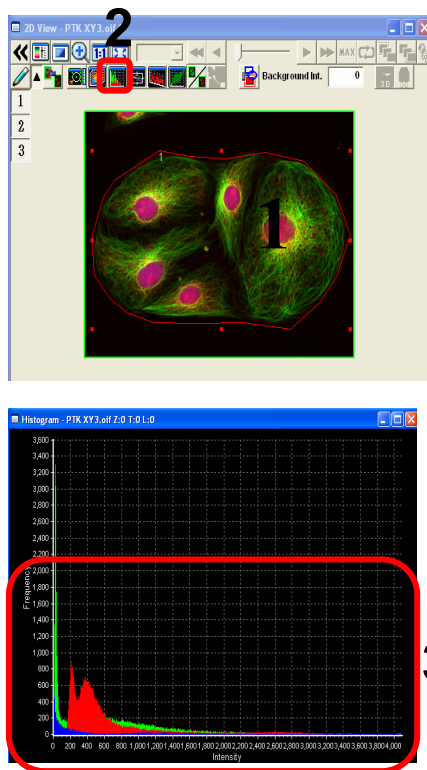
1. Line on the 2D image by ROI 

2. Click  “Intensity Profile”

3. “Intensity Profile” on the line is shown as intensity graph .

* State of colocalization between each Chs is figured out apart from intensity .

2D Image Analysis (Histogram)

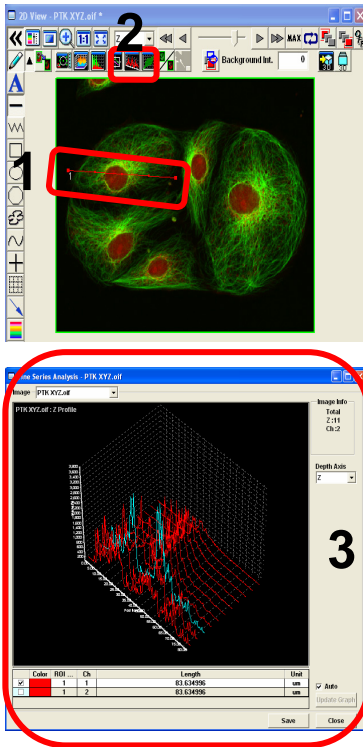


1. Enclose the region by ROI.


2. Click  “Histogram”

3. “Histogram” window is shown as a graph, frequency of intensity of each pixels is plotted on the region enclosed by ROI.

2D Image Analysis (Line Series Analysis)

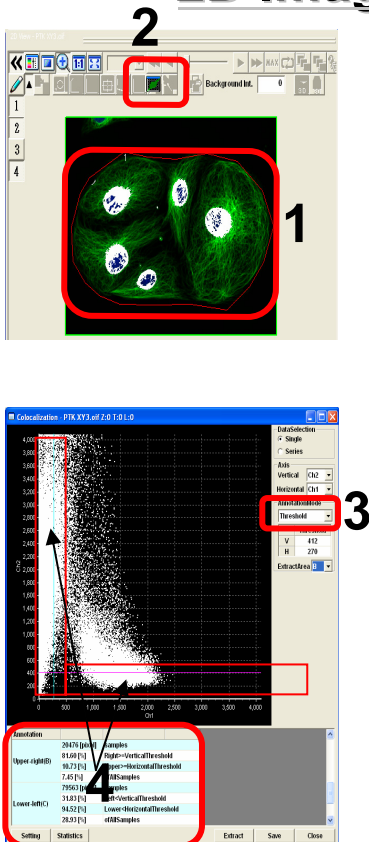


1. Line on the 2D image.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



1. Enclose an interesting region by ROI.

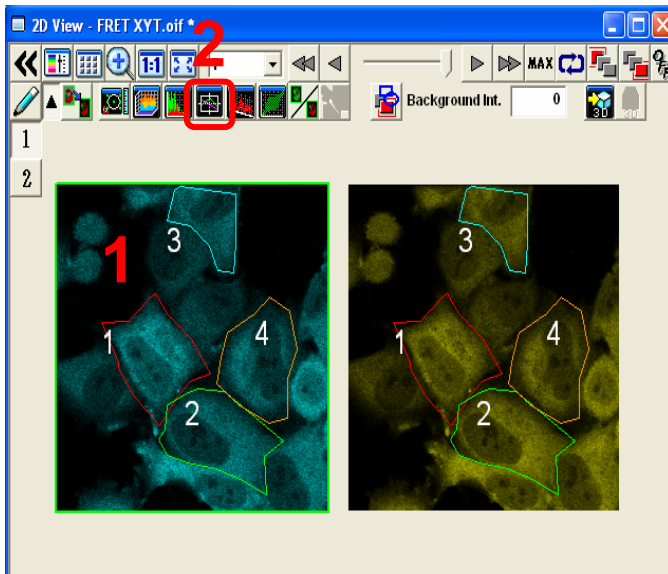
2. Click 

3. Select  **Threshold** from **Annotation Mode**.

4. According to move Thresholds of X,Y axis to right and left ,ups and down (**Enclose red color X,Y axis**), Co-localization result between 2ch is changed .

Information of Co-localization is listed under the scatter plot.

2D image Analysis(Series Analysis TimeLapse)



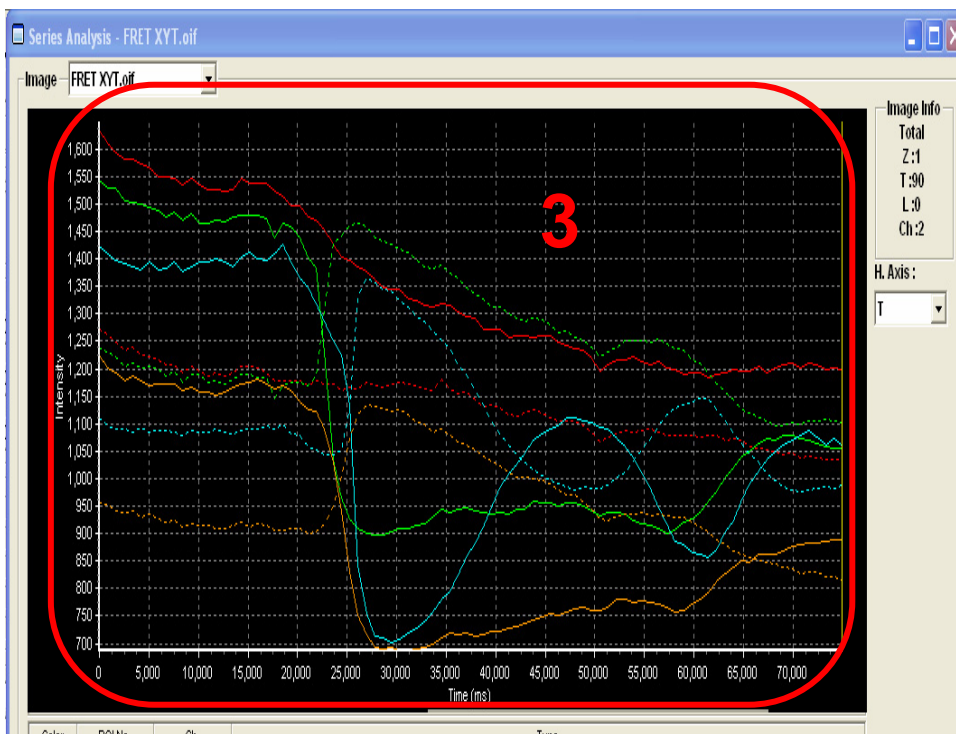
1. Enclose interesting regions by ROI

2. Click

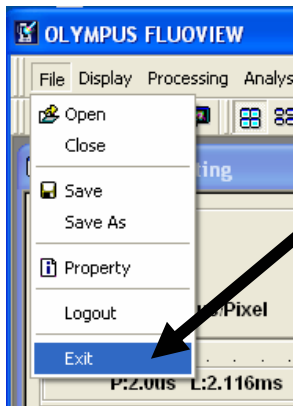


“Series Analysis”

3. “Series Analysis” graph is shown below, Y axis shows intensity, X axis shows time and then be able to see time series reaction each ROIs.



Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.

(2) On the Shut Down Window, select Shut Down and click on OK.

3. Turn the laser OFF.

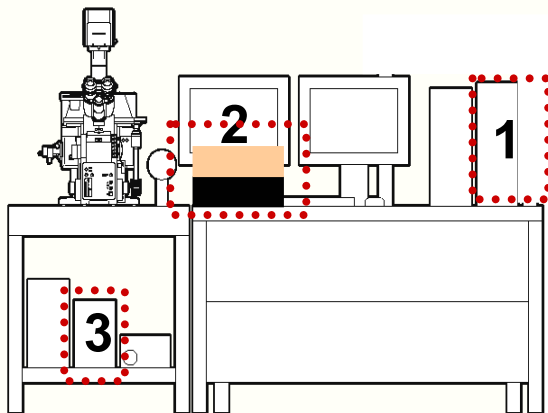
(Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF

4. Turn the mercury burner power OFF.



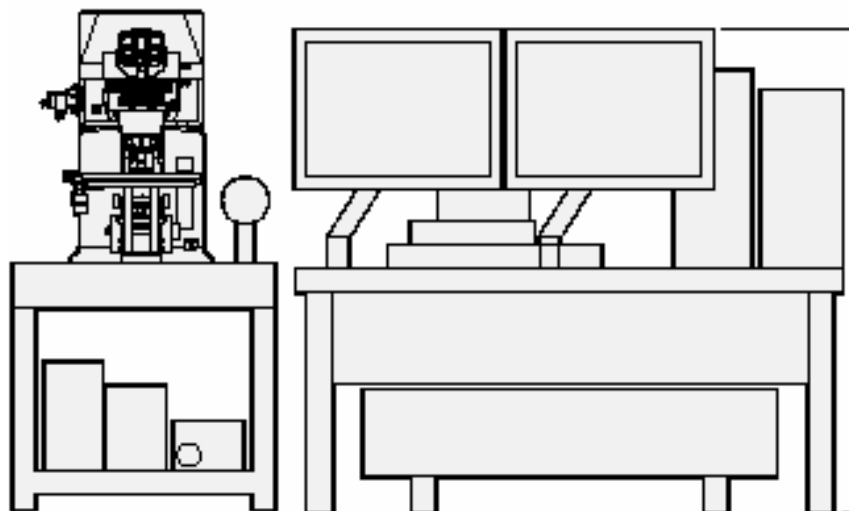


Laser Confocal Scanning Microscope

FV1000D Spectral Type

(Upright Microscope BX61)

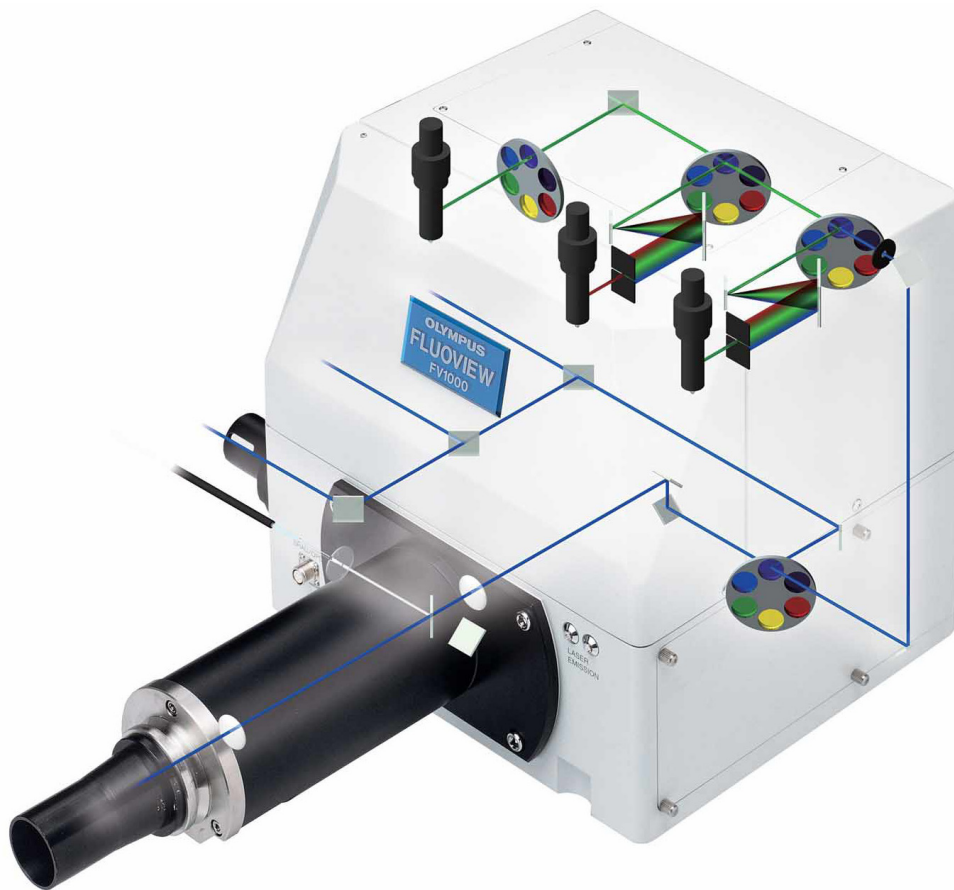
Operation Manual



Contents

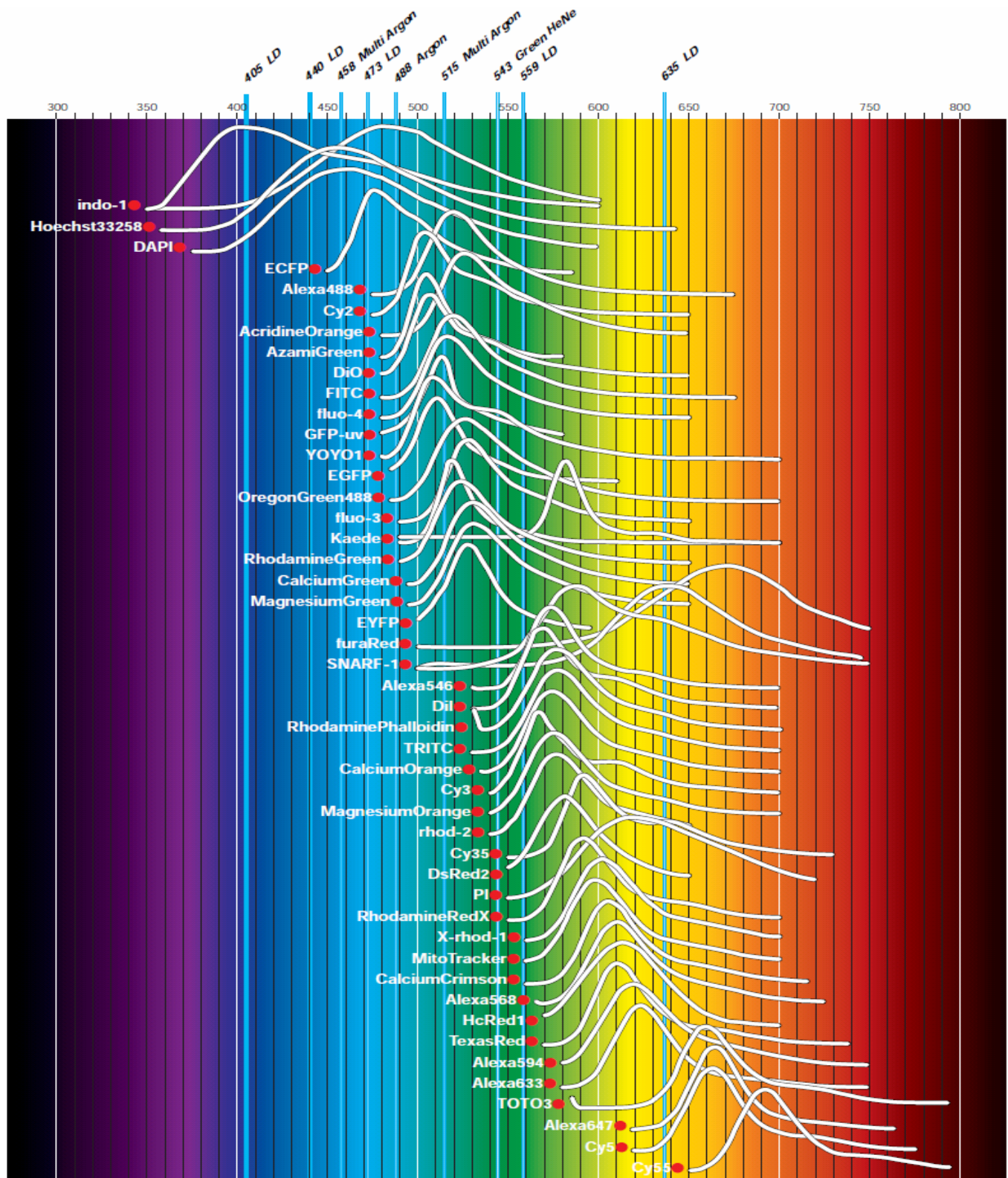
System introduction	-----	3
FV1000D Laser DyeList	-----	4
System Preparation	-----	5
Visible Observation		
Observation of Fluorescence image	-----	6
Observation of Differential Interference Contrast Images	-----	7
Image Acquisition		
Overview of Operation Panel for Image Acquisition	-----	8
Single Stain on XY Image		9-11
Complement of adjusting the image	-----	12-13
Double Stain on XY Image	-----	14-15
Sequential scan Line Sequential	-----	16
Double Stain on XYZ image	-----	17-18
Four Stain on XY Image	-----	19-21
Single Stain + DIC on XY Image	-----	22-23
Merge the image between fluorescent XY image and DIC image	----	24
Spectral Image on XYZ Image	-----	25-27
Unmixing	-----	28-30
Reload the image conditions	-----	32
Image Analysis		
Overview of the 2D Operation Panel / Opening a file	-----	33
Making 2D Z projection file Images	-----	34
Saving a Z section image as 2D image	-----	35
Inserting the Scale Bar	-----	36
Rotating a Three-dimensional Image	-----	37
Saving an Image	-----	38
Saving Rotating a Three-dimensional animation file	-----	39
2D Image Analysis		
Edit the image color and contrast	-----	40
The image of Z section	-----	41
Intensity Profile of each Z sections	-----	42
Measure	-----	43
Line Intensity Profile on the 2D image / Histogram	-----	44
Line Series Analysis / Co-localization	-----	45
Closing the System	-----	46

Spectral Type Main Scanner

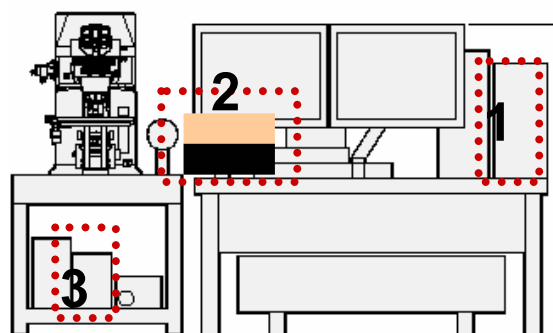


Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe (G) 543nm



System Preparation



Wait for a moment until the software is started


1. Turn the computer ON.
[In case of equipped concentrated power supply, power on it first]
2. Turn the laser ON
(Turning the key switch)
 - 2-1. LD559nm ON
 - 2-2. Multi Ar 458nm 488nm 515nm
 - 2-2. HeNe(G) (643nm) ON
3. Turn the mercury burner ON for Fluorescence observation.

4. Log on Windows

Enter Password ,Customer name is below

User name: Administrator

Password : fluoview

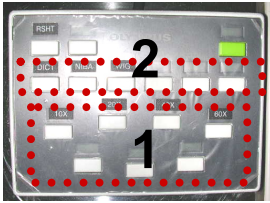
5.  Double click this icon to log on to ASW

User name: Administrator

Password : Administrator

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch




1. Select an objective lens by using the hand switch
2. Select fluorescent filter cube

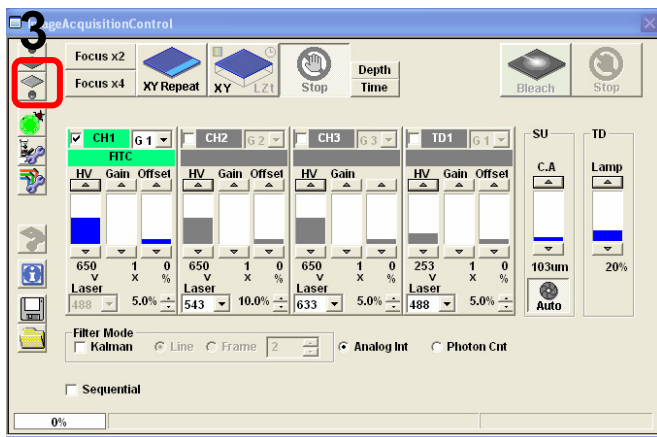
MEMO

Fluorescence filter

NIBA: Blue Excitation / Green Fluorescence
(Ex.: FITC, EGFP)

WIG: Green Excitation / Red Fluorescence
(Ex.: Rhodamine, DsRed)

3.  Click the button on the Fluoview software



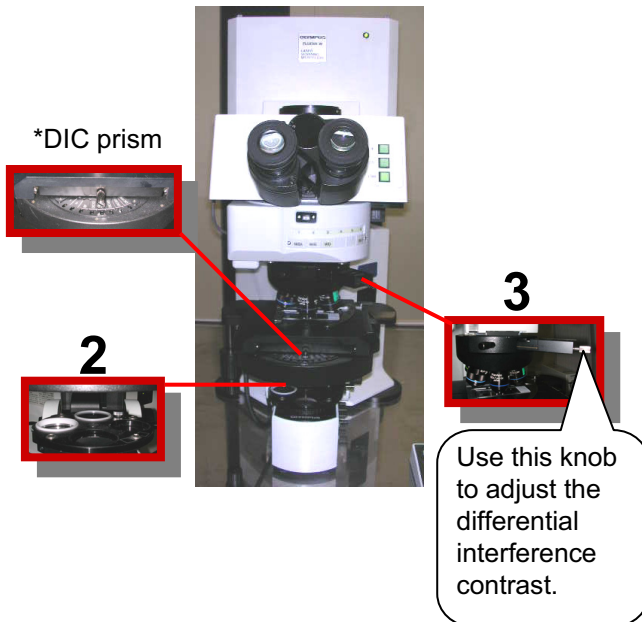
4. Focus to the specimen

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■




Hand switch

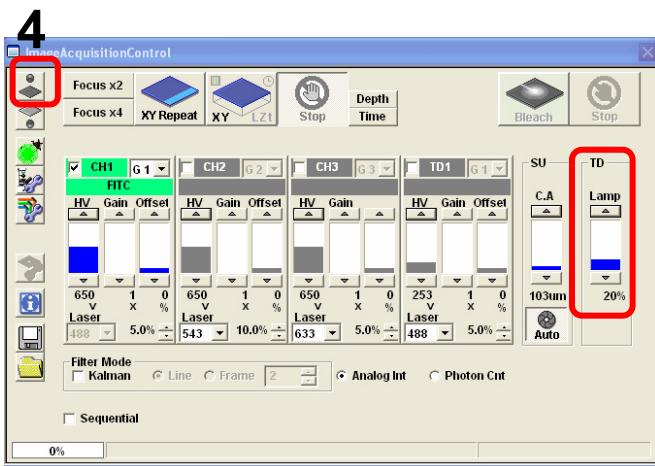


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software



5. Focus to the specimen

Overview of Operation Panel for Image Acquisition

The image displays three screenshots of the image acquisition software interface, with various components labeled and described.

AcquisitionSetting

- Scan mode
- Scan speed
- Number of pixels
- Zoom & Pan
- Laser output adjustment
- Objective lens
- Focus
- Time Interval & Time Number (for acquisition of XYT or XT image)

ImageAcquisitionControl

- Transmitted light observation (visual observation)
- Fluorescence observation (visual observation)
- DyeApply
- Optical path diagram
- TwinScanner setting
- Save acquisition conditions
- Load acquisition conditions
- Scan buttons
- Select XYZ, XYT or XYL
- Adjustment of each channel
- Confocal aperture
- Light intensity adjustment for halogen bulb
- Kalman

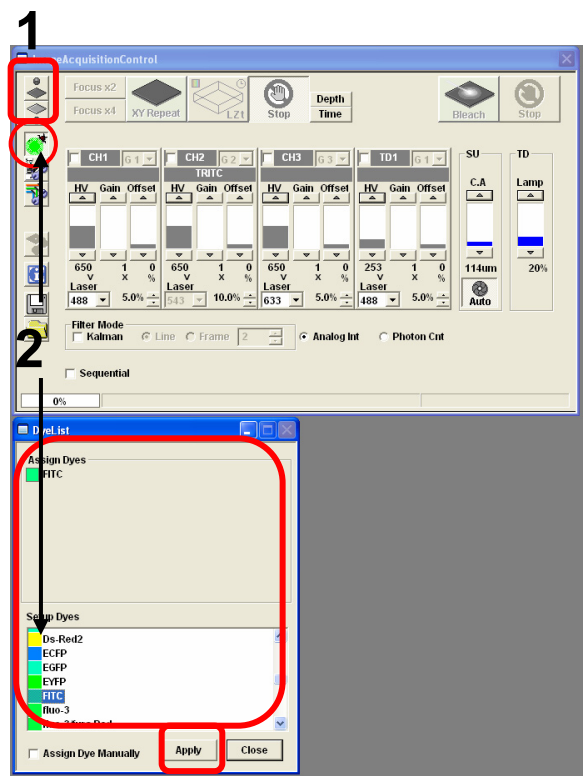
Image View



- Image display window
- Image file thumbnail
- Display of files in the memory

Image Acquisition (Single Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Single stain of green fluorescence dye (FITC)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

(The DyeList panel can be closed by using the Close button.)

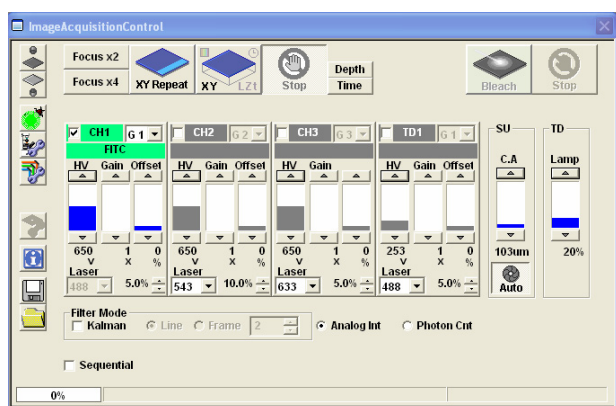
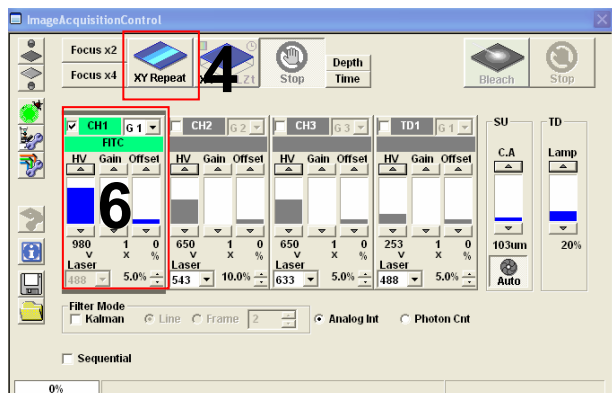


Image Acquisition (Single Stain on XY Image)



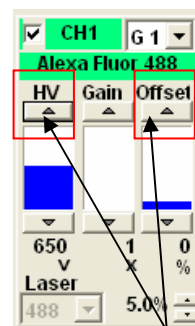
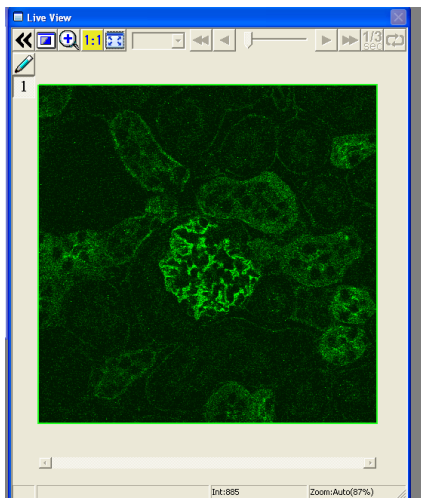
4. Press XY Repeat button click to get image



: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



· Adjust sensitivity of HV and reduce noise by offset

7. Press keyboard **Ctrl + H key**

Optimized PMT adjustment brightness intensity 2 color between white and black,

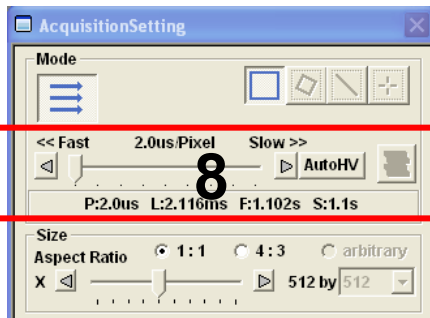
Maximum intensity is 4095 (12bit) if intensity is over 4095, color is changed to red (saturation)

* Basically, **Gain value is 1**



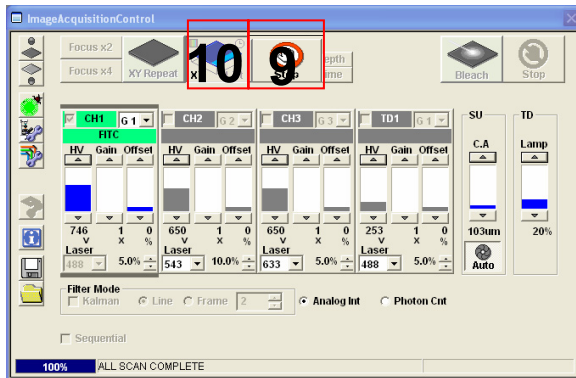
7


Image Acquisition (Single Stain on XY Image)




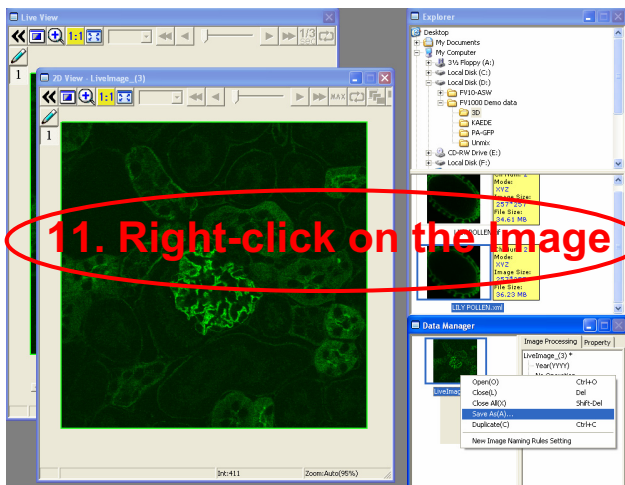
8. Select AutoHV and then select ScanSpeed.

*As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



9.  Press the Stop button to stop scanning.

10.  Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

Save the image as TIFF,BMP,JPEG format Select "Export" and chose the format TIFF, BMP, JPEG.

■Memo■

File formats specifically for the FV10-ASW

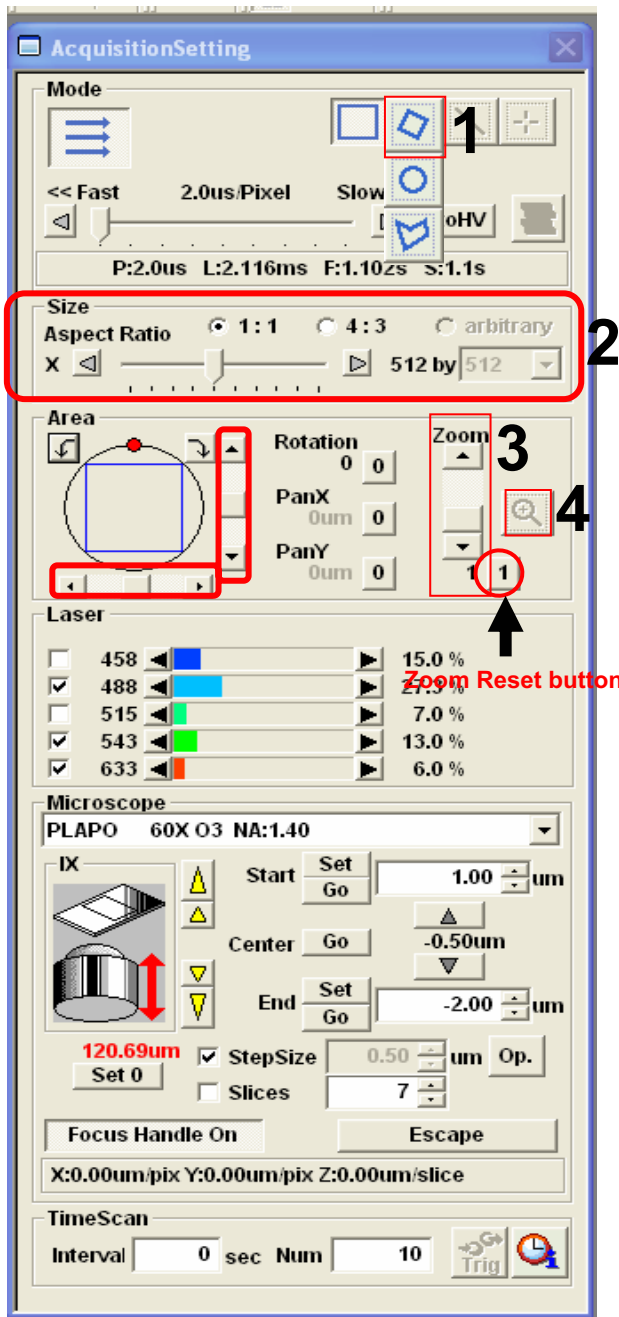
OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

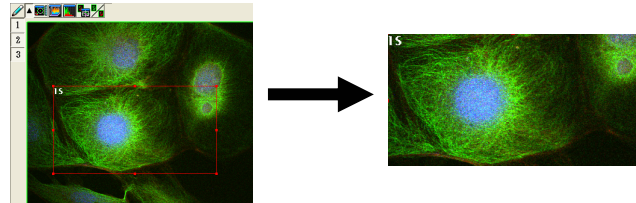
OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Complement of adjusting the image



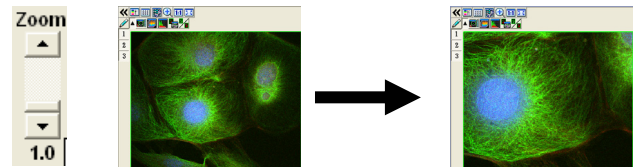
1. Click "Clip scan" button , and enclose an interesting region's image on the whole image.




2. pixel setting
*The standard pixel is 512 x 512

3. Zoom Setting

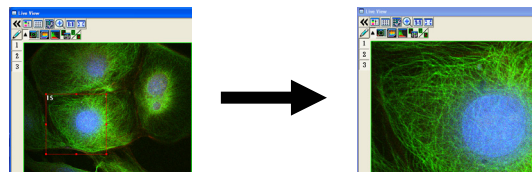
Press "XY Repeat" to scan and set zoom value.



Above image is zoomed From 1x to 2
* Scan speed and pixel resolution remain even zoom value is changed

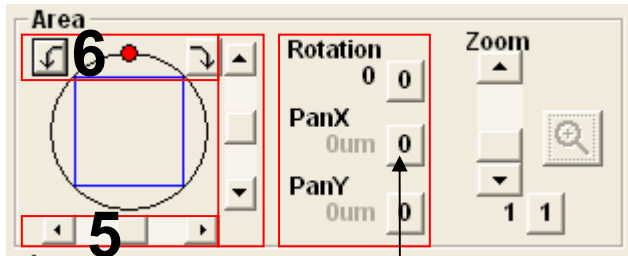
4. Click  Zoom scan, and be able to enclose an interesting region on the whole image

Press XYRepeat to scan after enclosing the area

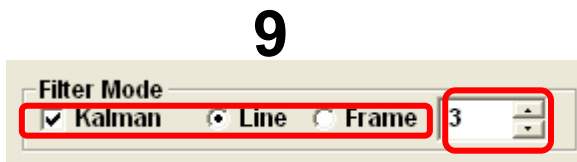
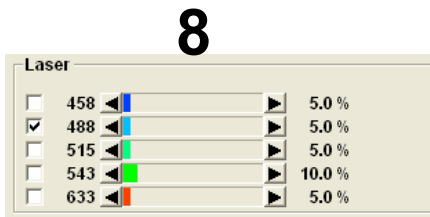


* Scan speed and pixel resolution remain even zoom value is changed

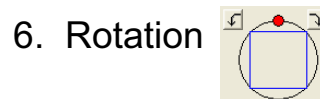
Complement of adjusting the image




PanX,Y and Rotation reset button



Be able to move the field of view to set Pan X,Y without stage action



Be able to rotate the whole image.

7.  Click "Auto" button to acquire Optimized Confocal aperture
Confocal aperture . . . change confocal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

8. Laser Intensity . . . More Laser intensity is increase , more bright image is .

* More increase laser intensity is , more discoloration image is .

9. Kalman accumulation . . . Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

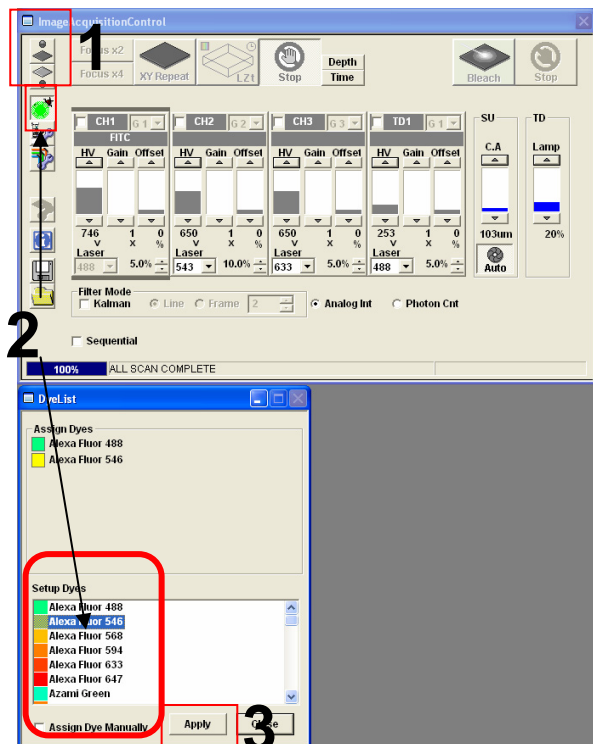
Disadvantage: Some blur occurs due to averaging of images.



Image Acquisition (Double Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



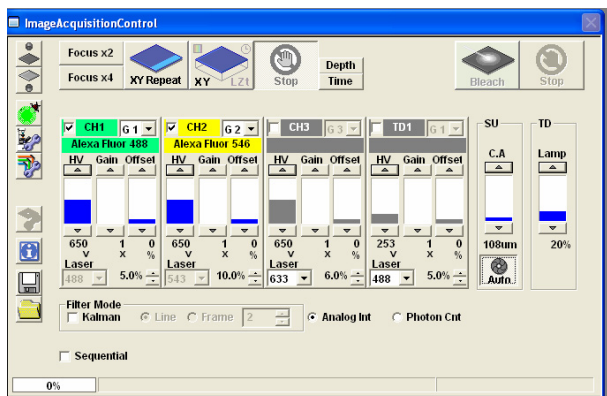
1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

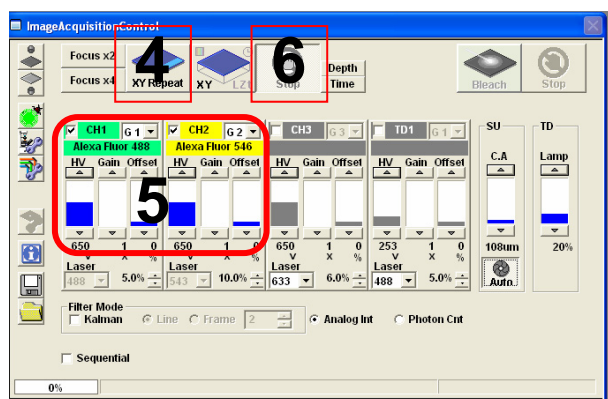
3. Click “Apply” button.

(The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

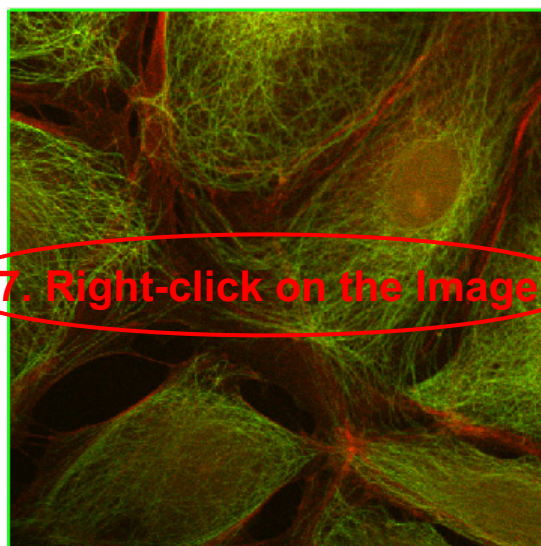
Image Acquisition (Double Stain on XY Image)



4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
(The image adjustment is outlined below.
For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)



■Memo■ Scan buttons



: Continuous scan



: Stop scan



: Rough scan (Line skipped)

7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

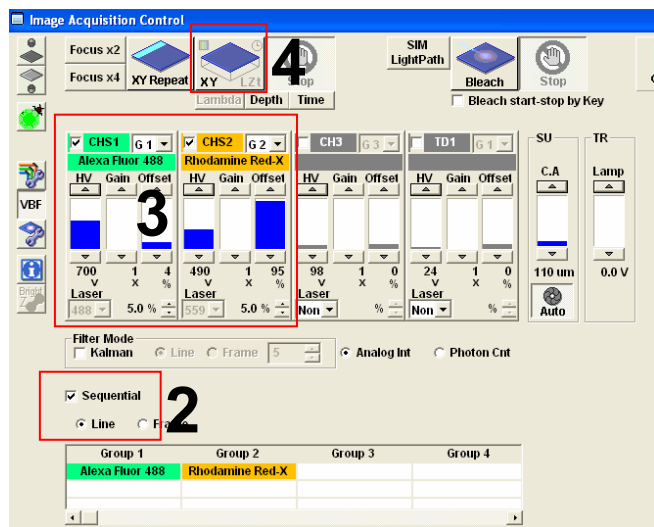
Image Acquisition

(Double Stain on XY Image)

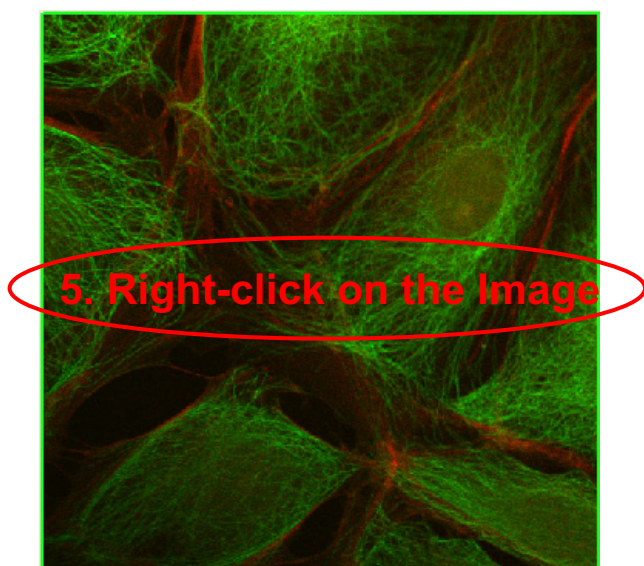
■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)



1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.
2. Check Sequential and select Line.
3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
4. Press the XY button to acquire an image.
5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.) The image is acquired.



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

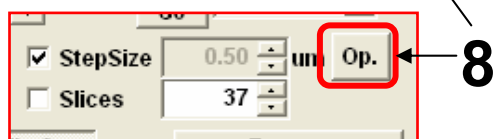
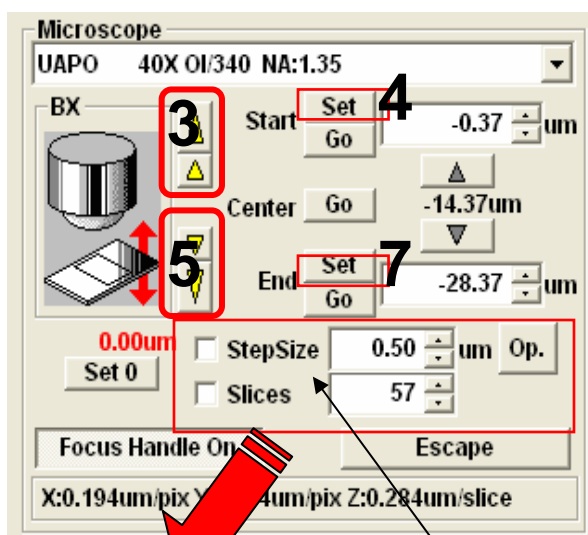
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Double Stain on XYZ Image)

■ Acquisition of 3D images (XYZ) (fluorescence image only) ■

Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



■Memo■

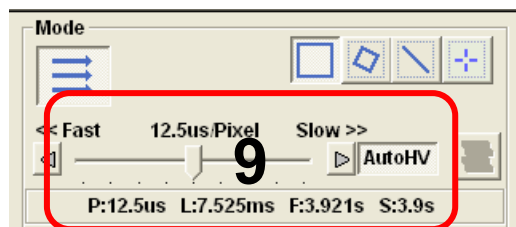
and buttons

: Moves 1.0μm with a single click.

: Moves 0.1μm with a single click.

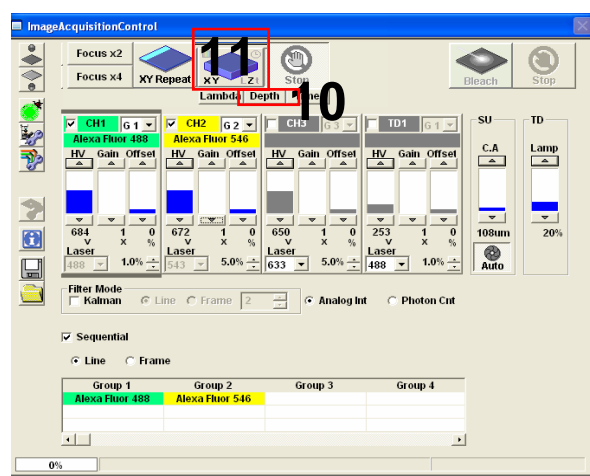
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

Image Acquisition (Double Stain on XYZ Image)



9. Select AutoHV and then select ScanSpeed.

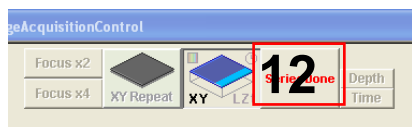
10. Select Depth.



11. Press the XYZ button to acquire an image.

12. Click on "SeriesDone", and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

13. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

13. Right-click on the Image

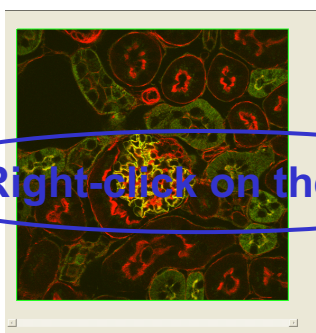
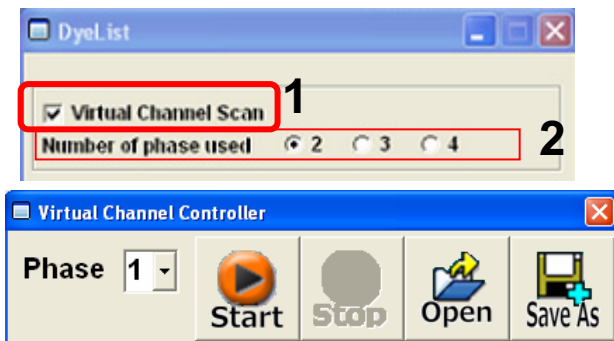


Image Acquisition (Four Stain on XY Image)

■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■

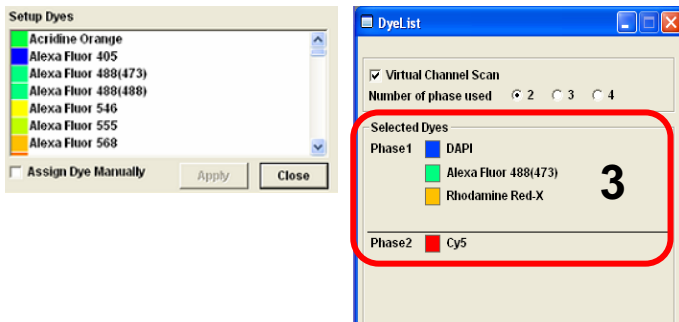
Sample: Four stain of Blue fluorescence dye (DAPI), green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

This is the procedure to acquire images through Virtual Channel scan

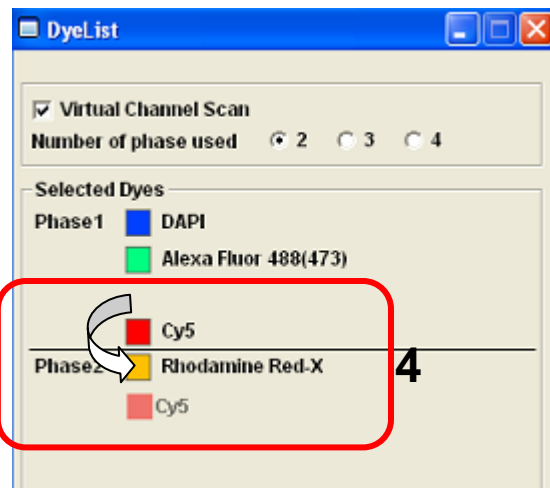


1. ☒ Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then **“Virtual Channel Controller”** is automatically turned on.

2. Select a number of Virtual Channel from **“Number of phase used”**.



3. Select 4dyes from DyeList 4th dye is registered in **“the Phase 2”**.



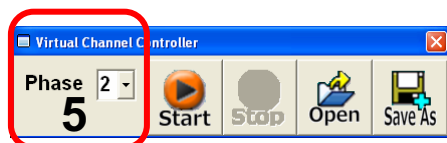
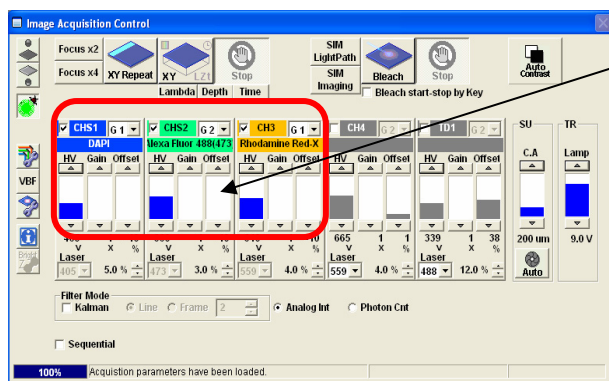
* RodaminRed is able to be registered on **“Phase2”** to drag.

Image Acquisition (Four Stain on XY Image)



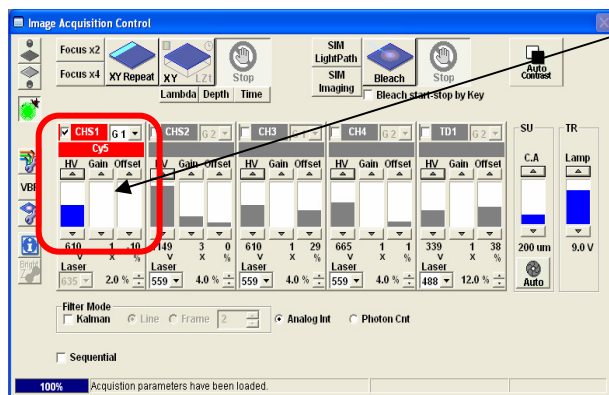
4. Select "Phase1", "DAPI", "Alexa488", "RhodaminRed" are registered on ImageAquisitionControl.

* Slit and Filter, DM are automatically set for "DAPI" "Alexa488" "PhodaminRed"



5. Select "Phase2", Cy5 is registered on ImageAquisitionControl

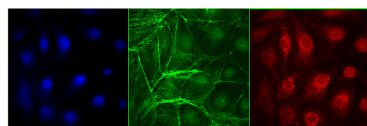
* Slit and Filter, DM are automatically set for "Cy5"



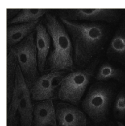
Adjust the image at each phases


"Phase1"

"Phase2"



6




6. Adjust the image to click  "XY Repeat" at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

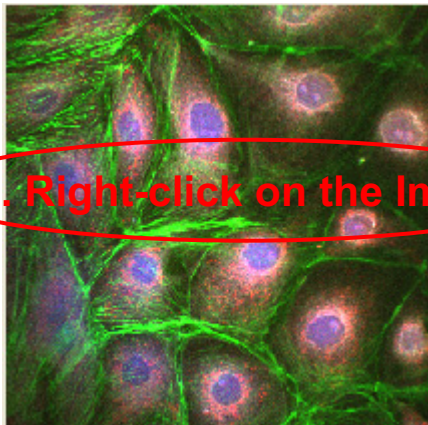
Image Acquisition (Four Stain on XY Image)



7

7. Click  on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

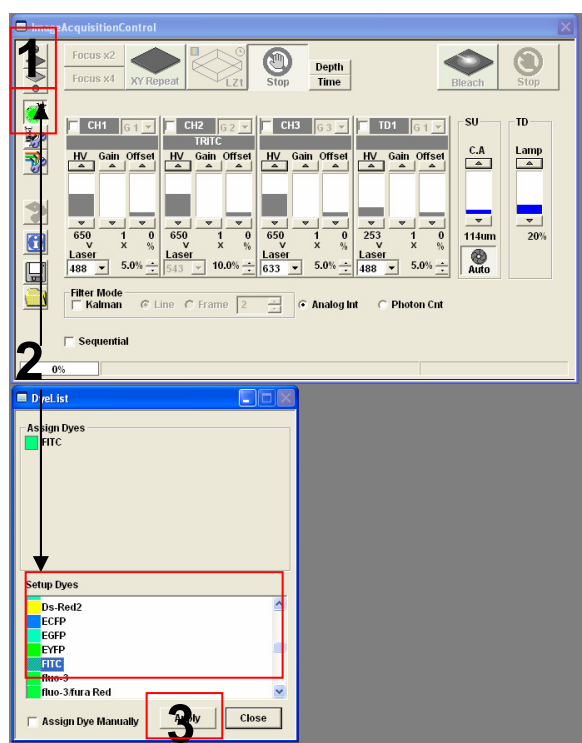
OIB format:



Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Single Stain + DIC on XY Image)

■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■

Sample: Green fluorescence dye (FITC) and differential interference contrast image

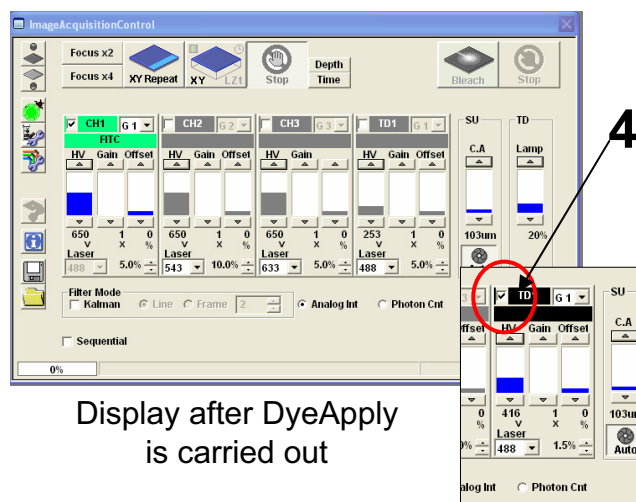


1. Click on the FV10-ASW software  button to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

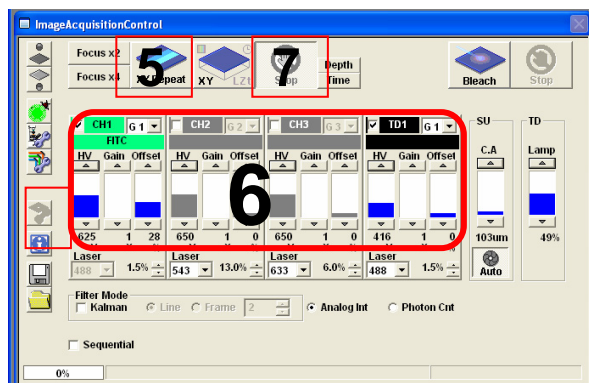
3. Click on the Apply button.
(The DyeList panel can be closed by using the Close button.)



Display after DyeApply
is carried out

4. Check TD1.

Image Acquisition (Single Stain + DIC on XY Image)



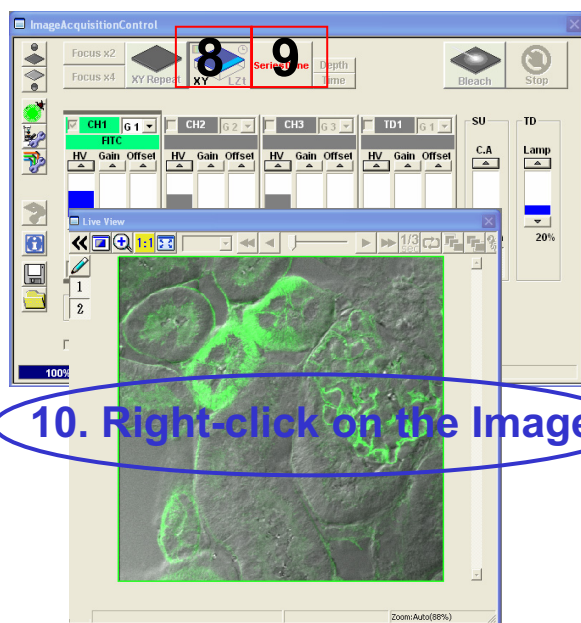
5. Press the “XY Repeat” button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the “Stop button” to stop scanning.

8. Press the “XY button” to acquire an image.

9. Click on “SeriesDone”, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



10. Right-click on the Image

10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

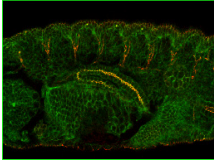
Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

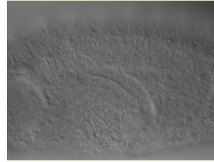
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Merge the images between fluorescent XY image and DIC image

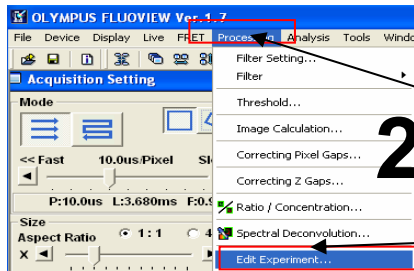
Edit different each files to the same file. This is available for making merge image
Between fluorescent image and focused DIC image.



1

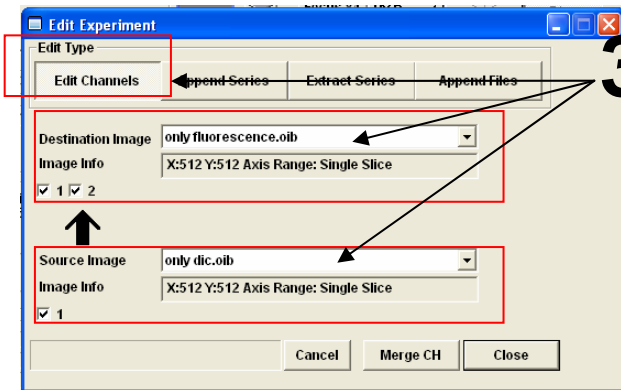


1. Open fluorescent image and
DIC image.



2

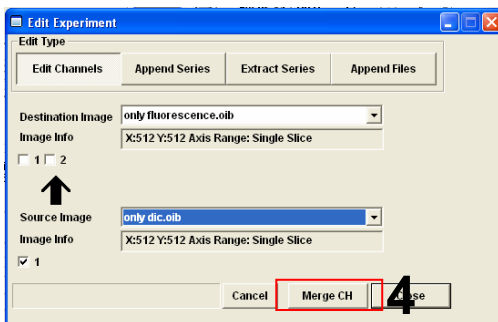
2. Select
Edit experiment from **Processing**



3

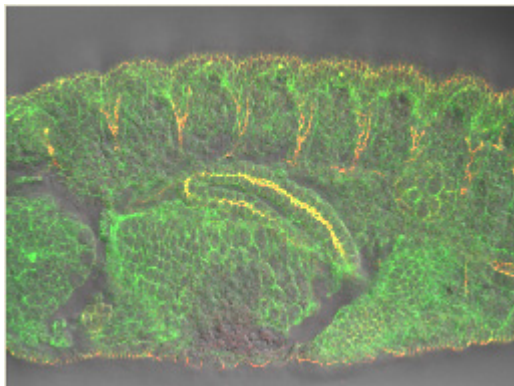
3. Click **Edit Channels**, and select
fluorescent image file at
Destination Image, select DIC
image at **Source Image**.

* Check Image Info ☒ 1 ☒ 2 to make the.
merge file. If all channels are checked, all
channels are reflected in the new merged file.



4

4. Click **Merge CH**, and then
the fluorescent image and the DIC
image are merged as the new file .



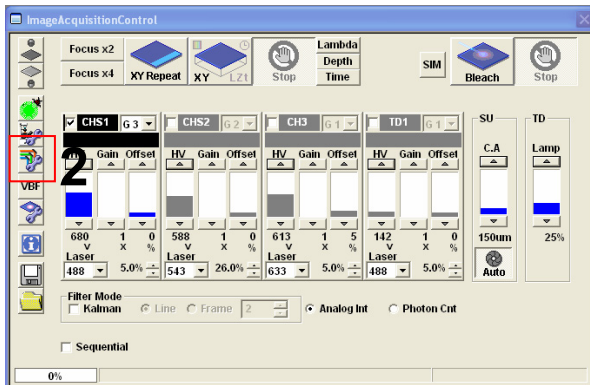
5



5. Merged image between the
fluorescent image and the DIC
image.


Image Acquisition (Spectral Image on XYL Image)

■ Acquisition of a spectral image (XYL) ■

Sample: Double stain of green fluorescence dye (Alexa Fluor 488)
and green fluorescence dye (YOYO-1)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.
Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the  button to view the optical path diagram.

3

3. Make settings as shown below.

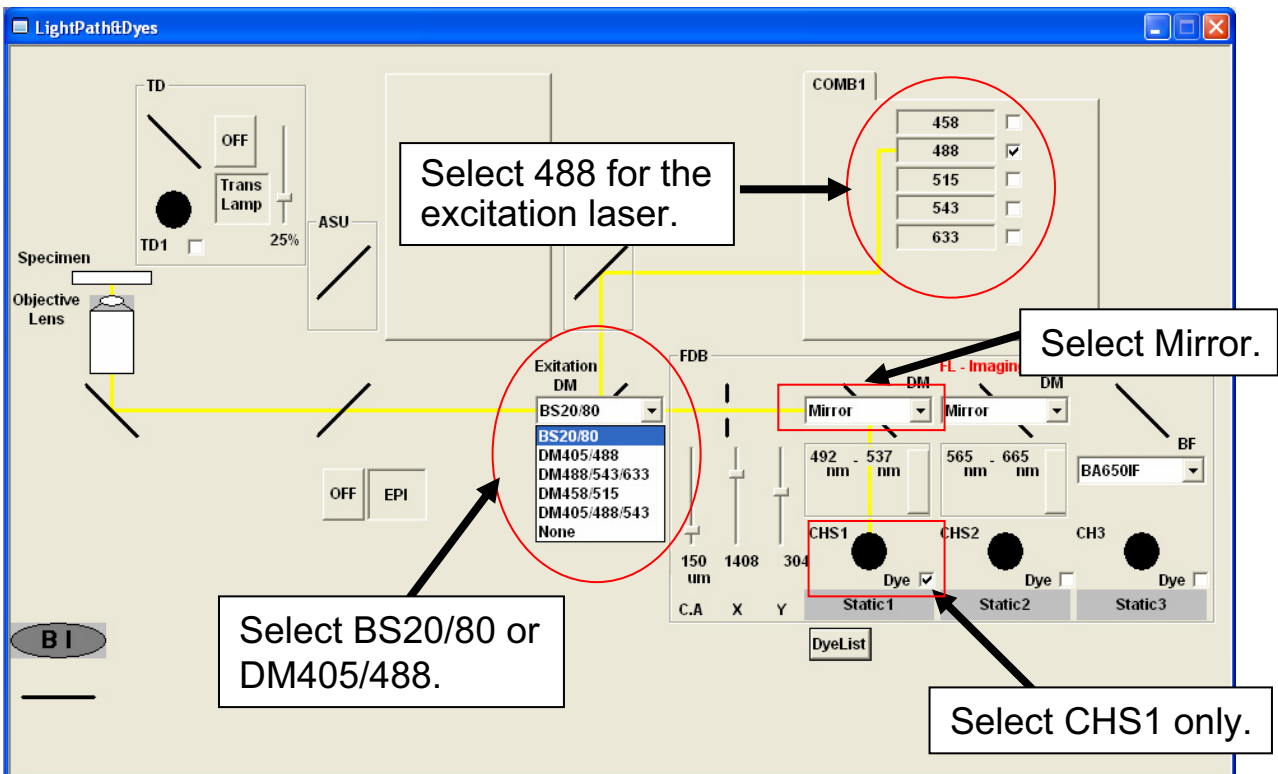
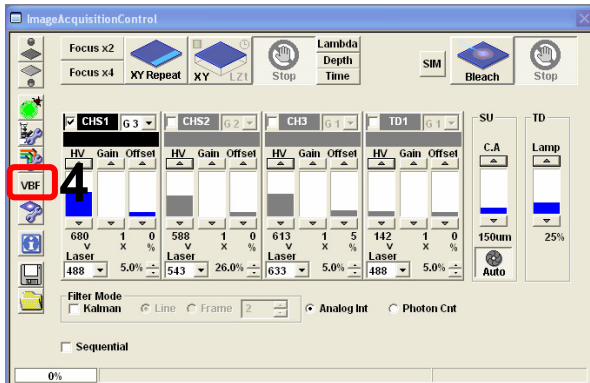
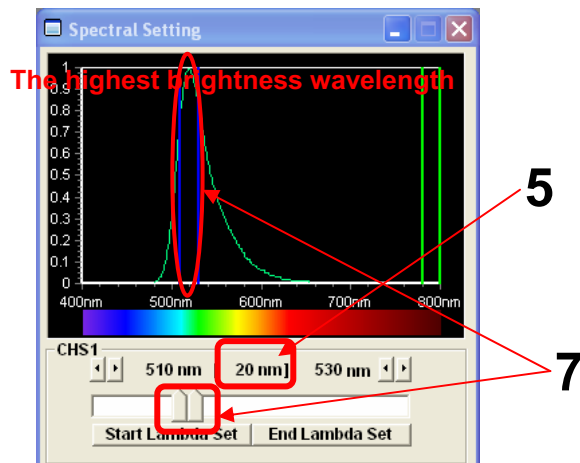


Image Acquisition (Spectral Image on XYL Image)




4. Click on the  button, and the Spectral Setting window appears.

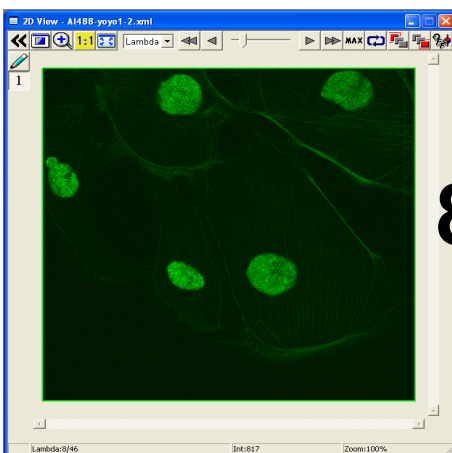
5. Set the slit width for CHS1 to 20 nm, for example.



6. Press the XY Repeat button to start scanning.

7. While observing the image, Click the left side of slit  and drag to the point which the highest brightness is achieved.

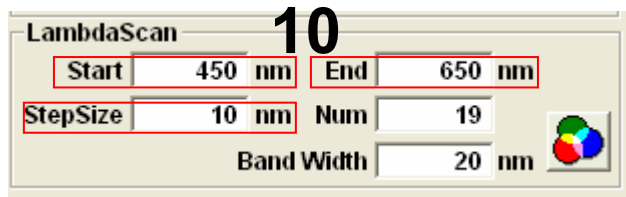
Note: Move the slit position only while keeping the slit width at 20 nm.



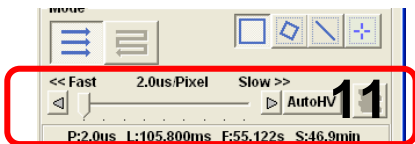
8. Adjust the image on the highest brightness.

9. Press the Stop button to stop scanning.

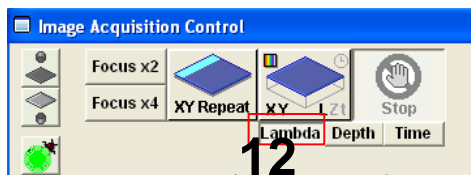
Image Acquisition (Spectral Image on XYL Image)




10. Set the range of wavelength to be acquired, the slit width and the step.
- Start = Start wavelength
 - End = End wavelength
 - Resolution = Slit width
 - StepSize = Step

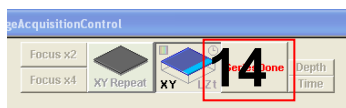


11. Select AutoHV and then select ScanSpeed.
- *As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



12. Select Lambda.

13.  Press the XYZ button to acquire an image.



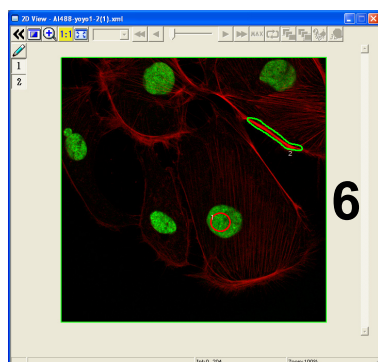
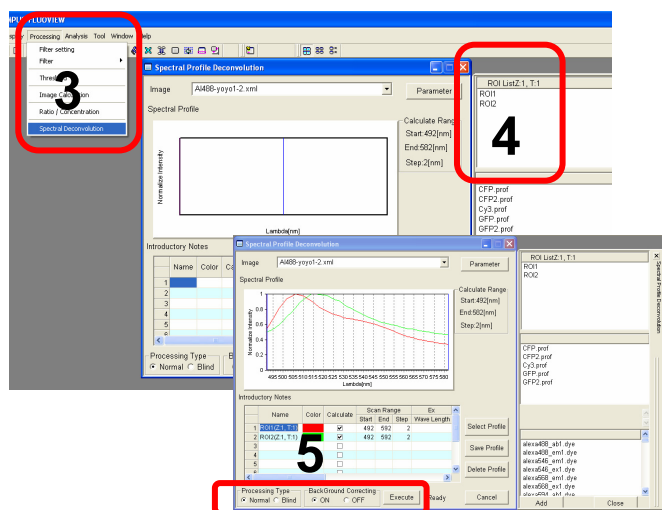
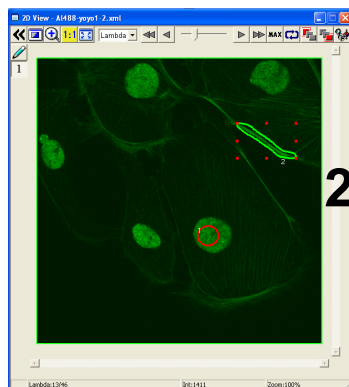
14. Click on SeriesDone, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.

Image Analysis (Unmixing)

I. When each fluorescence dye point is clear

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, derive the fluorescence spectrum for each fluorescence dye and obtain an unmixed image based on the fluorescence spectrums.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



Unmixed image

1. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.
2. Enclose a point dyed with Alexa Fluor 488 only and a point dyed with YOYO1 only.
3. From Processing on the menu bar, select Spectral Deconvolution.
4. Double-click on ROI1 and ROI2.
5. Check that the Processing Type is set to "Normal" and click on Execute.
6. An unmixed image is obtained.

Indicates channel assignments of unmixed images

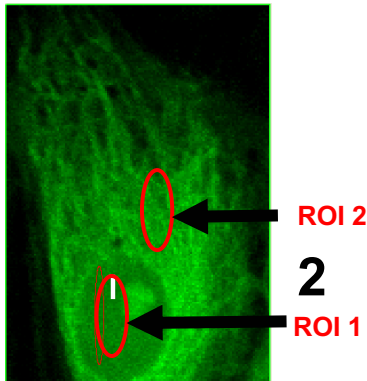
Introductory Notes				
	Name	Color	Calculate	
1	ROI1(Z:1, T:1)	Red	<input checked="" type="checkbox"/>	
2	ROI2(Z:1, T:1)	Green	<input checked="" type="checkbox"/>	
3			<input type="checkbox"/>	
4			<input type="checkbox"/>	

Image Analysis (Unmixing)

I. When each fluorescence dye point is clear

Sample: single stain of green fluorescence dye (GFP)
and auto fluorescence from cell

1



1. Open the XYL image (GFP + auto fluorescence).

2. Enclose a point dyed with GFP only and a point dyed with auto fluorescence only.

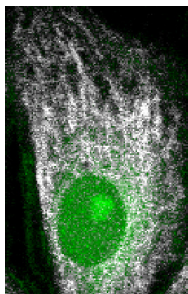
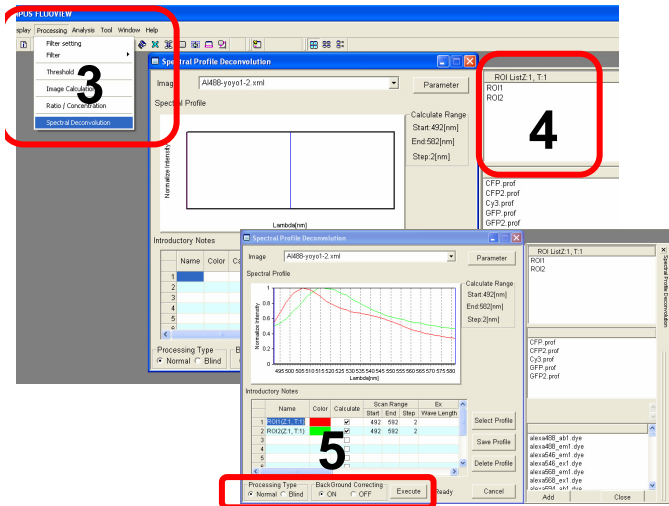
3. From Processing on the menu bar, select Spectral Deconvolution.

4. Double-click on ROI1(GFP) and ROI2(Auto fluorescence).

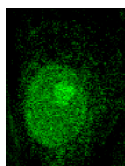
5. Check that the Processing Type is set to "Normal" and click on Execute.

6. An unmixing image is obtained.

Green color is GFP.
Gray color is Auto fluorescence.



6



Only GFP image

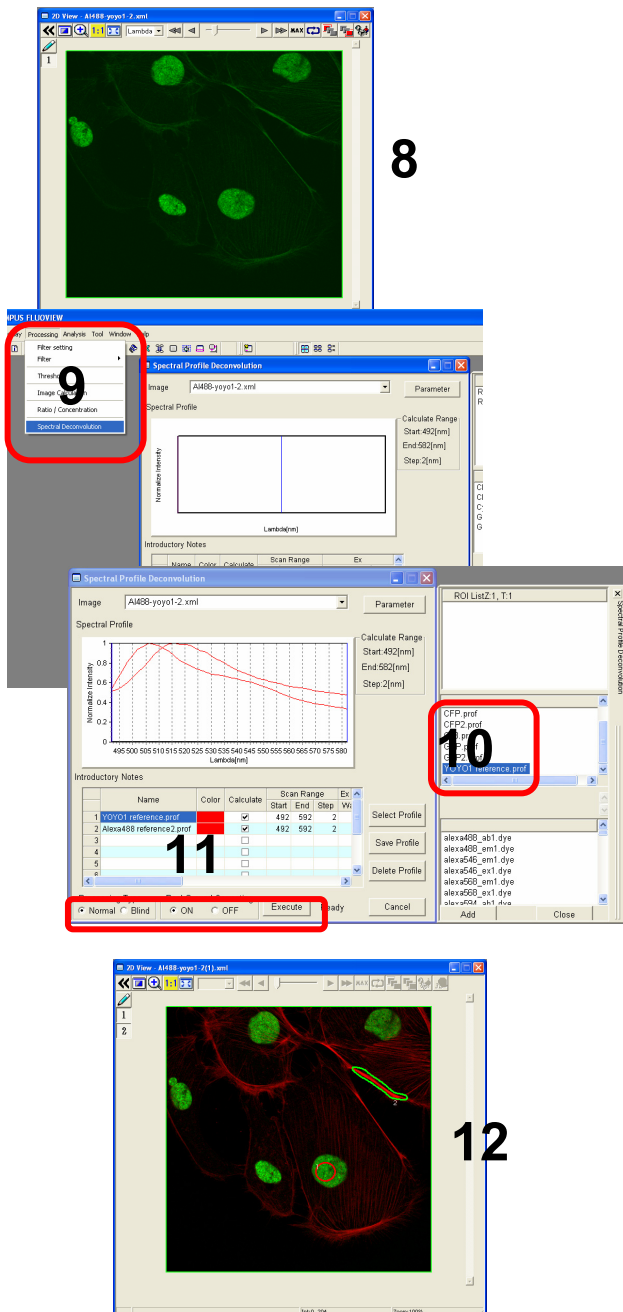
Unmixing image between GFP and Auto fluorescence

Image Analysis (Unmixing)

II. When a control sample is used

From an XYL image with a single type of fluorescence dye, derive the fluorescence spectrum of the dye and obtain an unmixed image based on the fluorescence spectrum.

Sample: Double stain of green fluorescence dye (Alexa Fluor 488) and green fluorescence dye (YOYO-1)



8. Open an XYL image file with both Alexa Fluor 488 and YOYO1 applied.

9. From Processing on the menu bar, select Spectral Deconvolution.

10. Double-click on Alexa Fluor 488 and YOYO1 (which have been registered) in the database of fluorescence spectrums.

11. Check that the Processing Type is set to "Normal" and click on Execute.

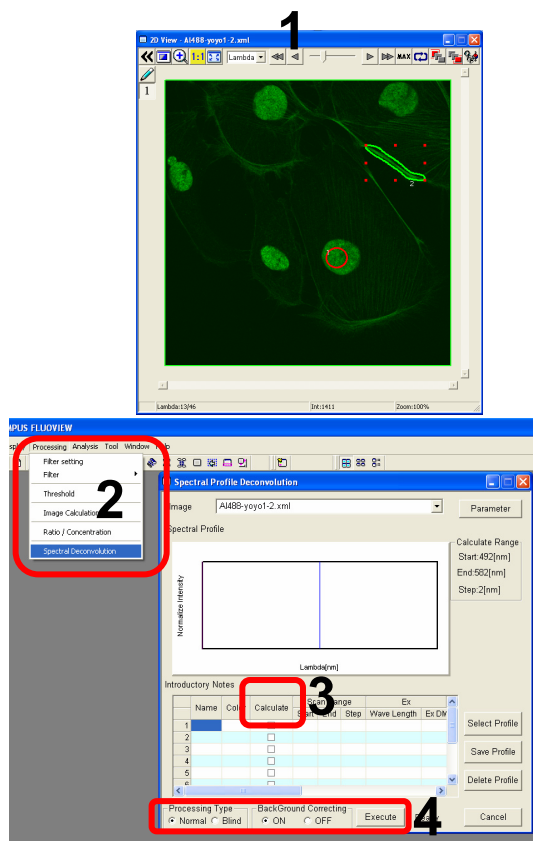
12. An unmixed image is obtained.

Image Analysis (Unmixing)

III. When only the number of types of fluorescence dyes is known (Blind Unmixing)

From an XYL image where fluorescence dyes with similar fluorescence spectrums are present together, obtain an unmixed image based on only the number of types of fluorescence dyes.

Sample: Sample with two unknown types of fluorescence dyes

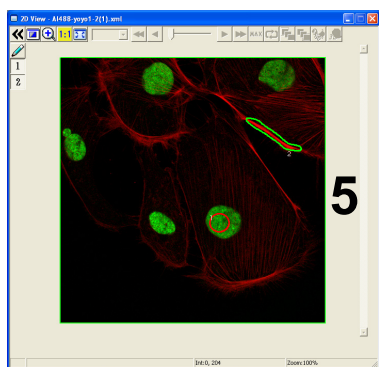


1. Open an XYL image file for a sample that has two unknown types of fluorescence dyes.

2. From Processing on the menu bar, select Spectral Deconvolution.

3. Click on two Calculate check boxes. (Click on three boxes when three types of fluorescence dyes are used.)

4. Check that Processing Type is set to "Blind" and click on Execute.

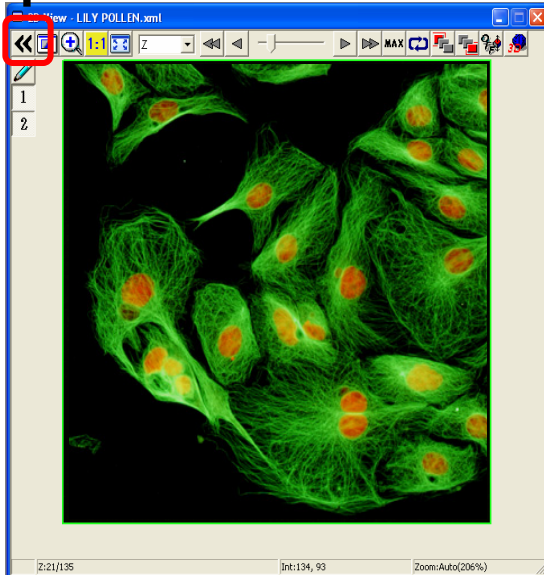


Unmixed image

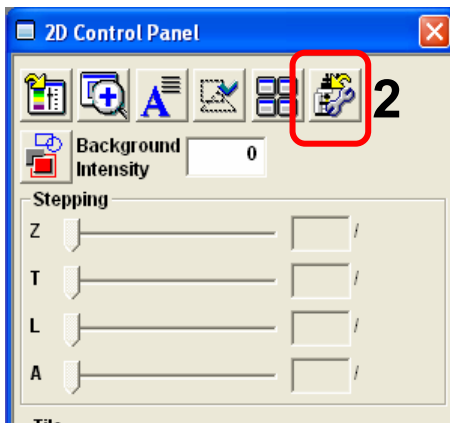
5. An unmixed image is obtained.

Reload the image conditions

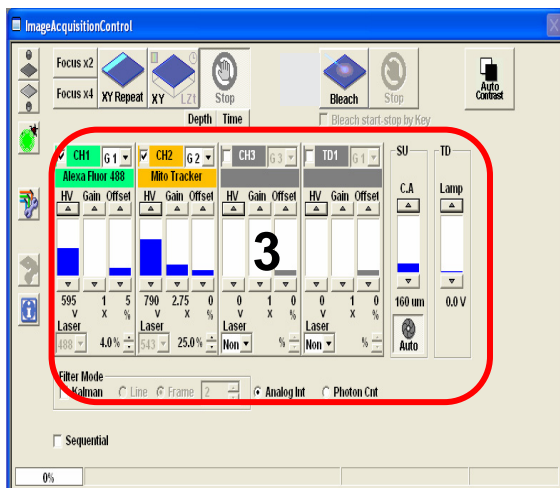
1



1. Open the file and click



2. Click



3. The conditions (HV,Offset, CA and so on) are reloaded .

Overview of the 2D Operation Panel

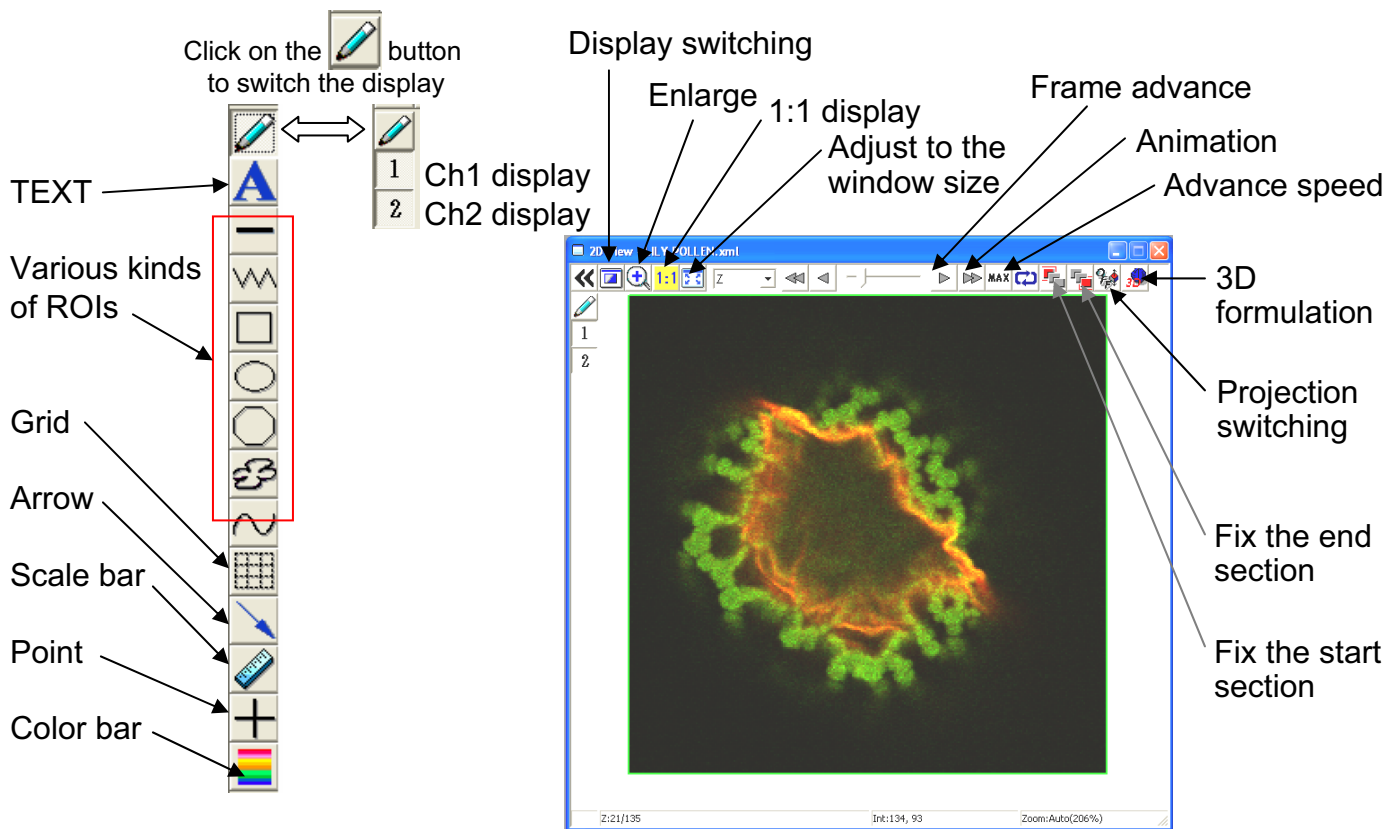
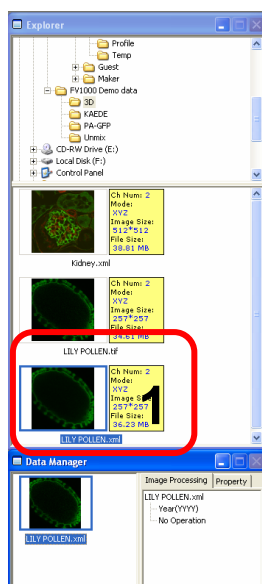
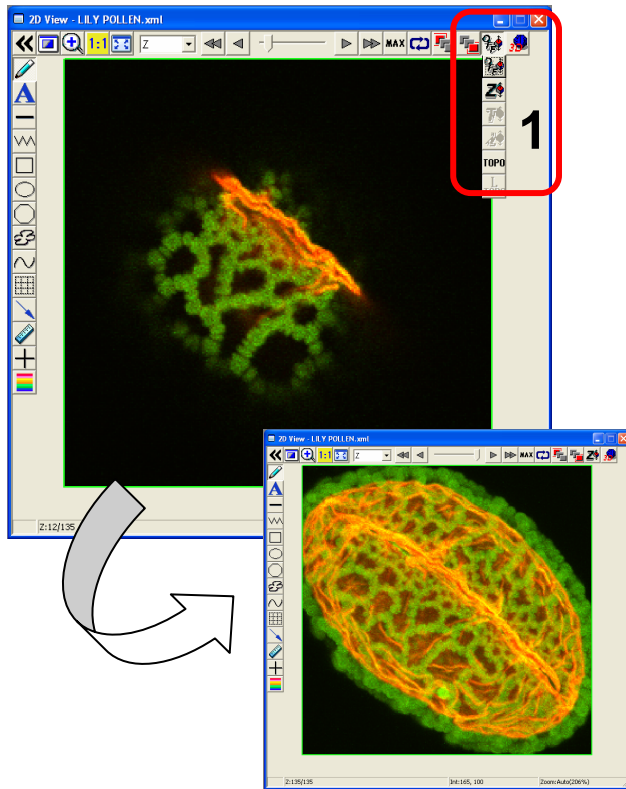


Image Analysis (Opening a File)

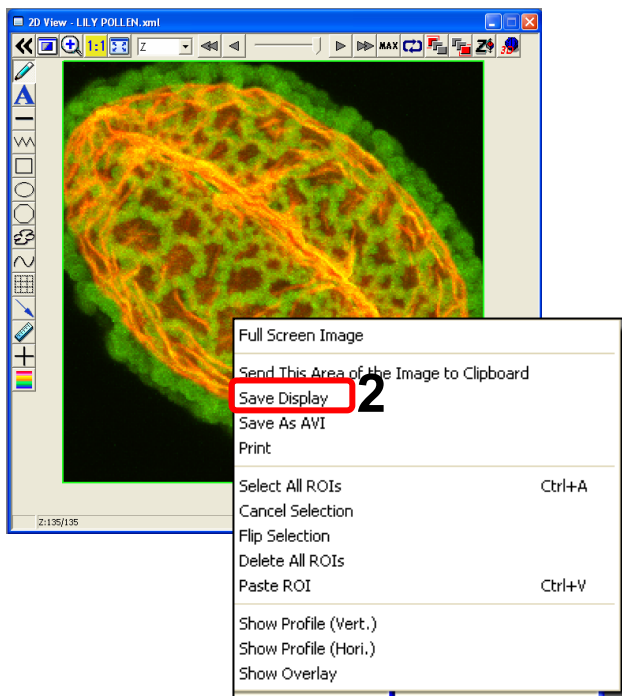


1. Double-click on a file to be opened from Explorer.

Image Analysis (Acquire a Projection Images)



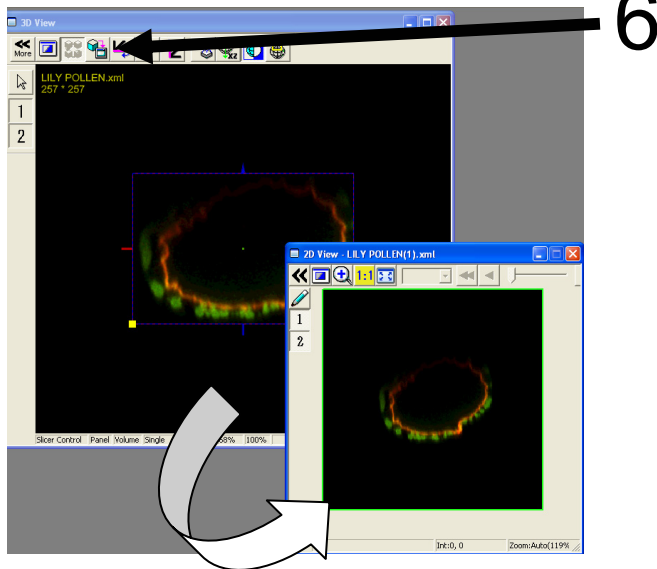
1. Click on the  button to select .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

Image Analysis

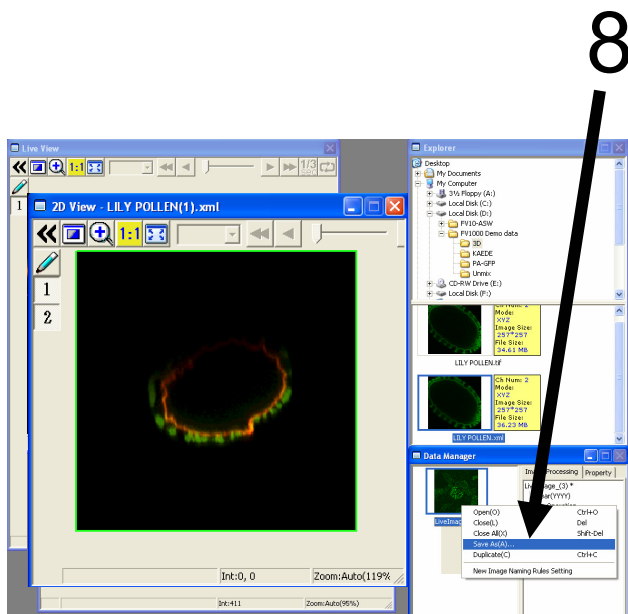
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

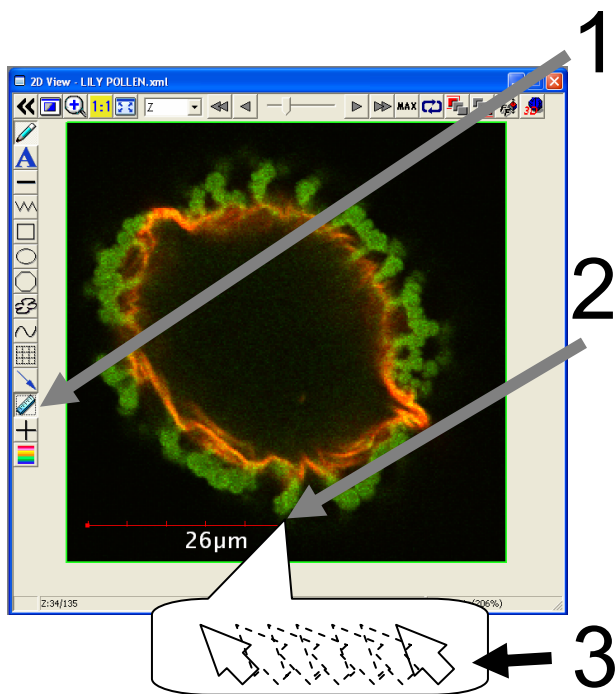
OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Analysis (Inserting the Scale Bar)

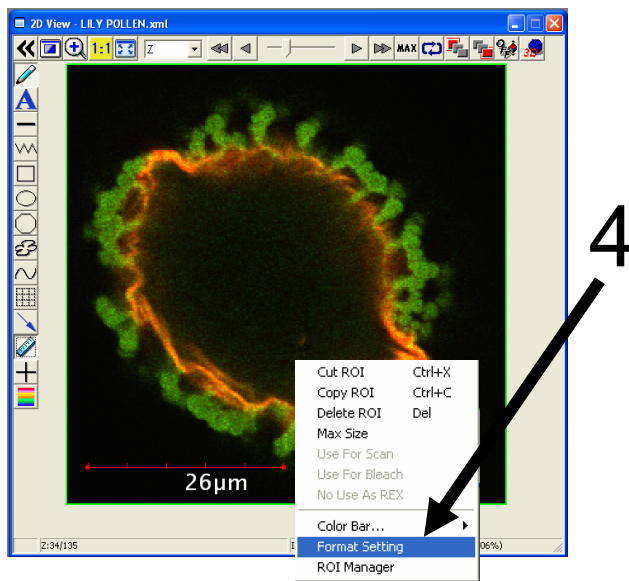


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

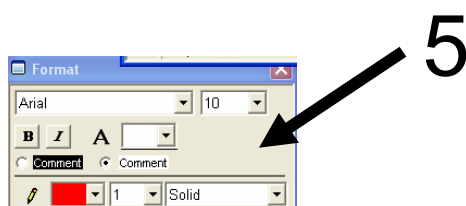
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

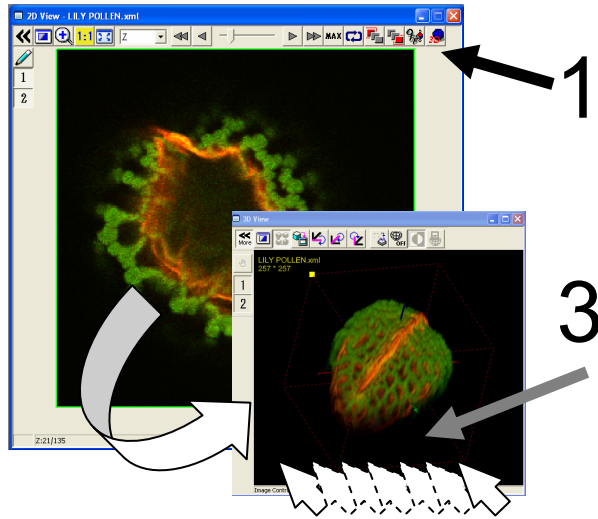
4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.




5. Change the setting in this window as required.

Image Analysis


(Rotating a Three-dimensional Image)




1. Click on the  button for a 2D View-(file name) image.
2. A 3D view is created.
3. Drag the mouse on the image to observe it at a certain angle.

Simple animation

4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

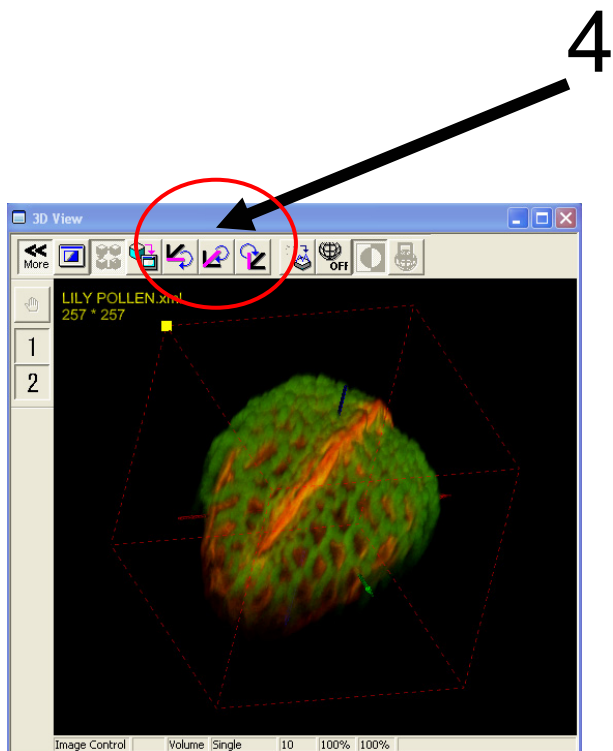
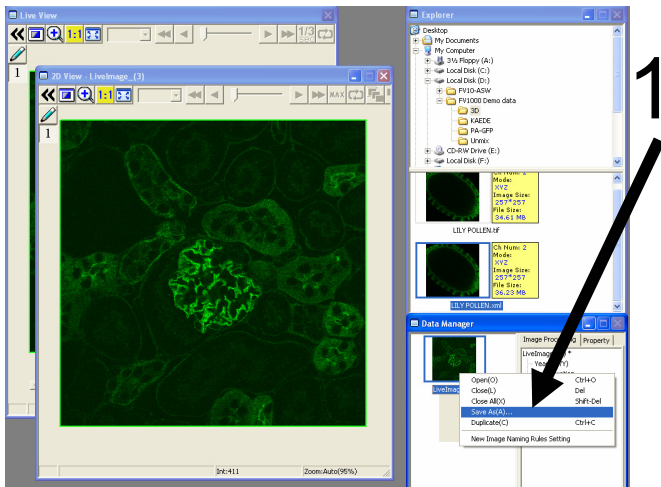


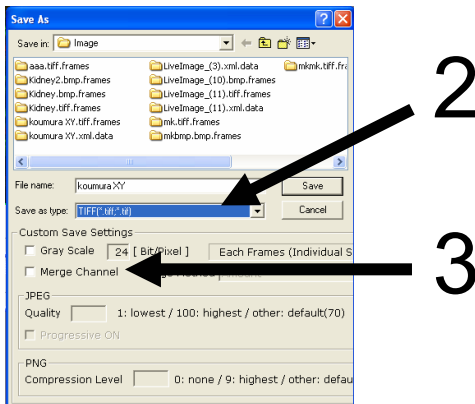
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB color.
4. Save the image.

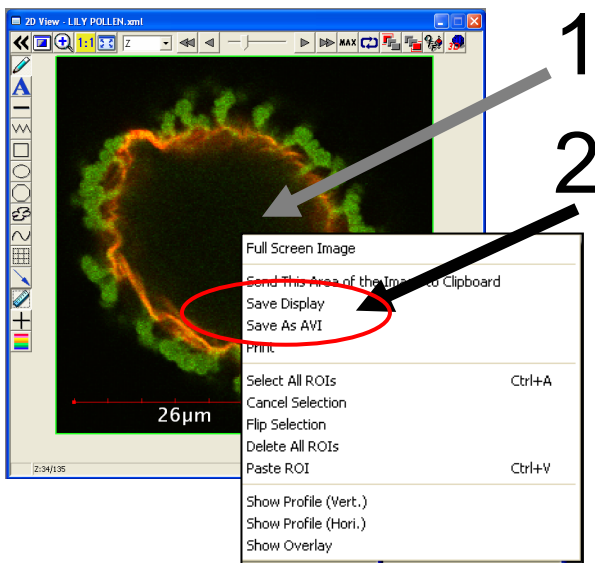
* BMP and JPEG formats are also selectable.



Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.



Convert an image with the scale bar inserted into a BMP format

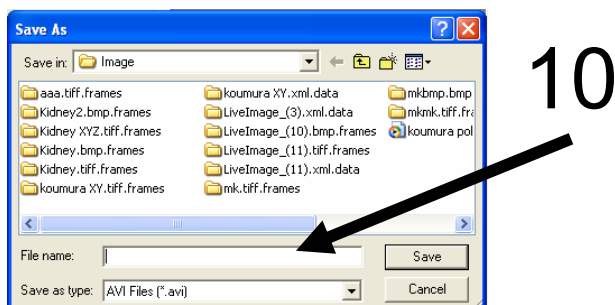
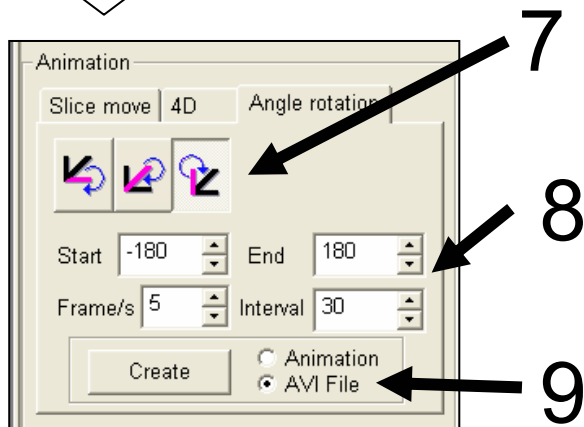
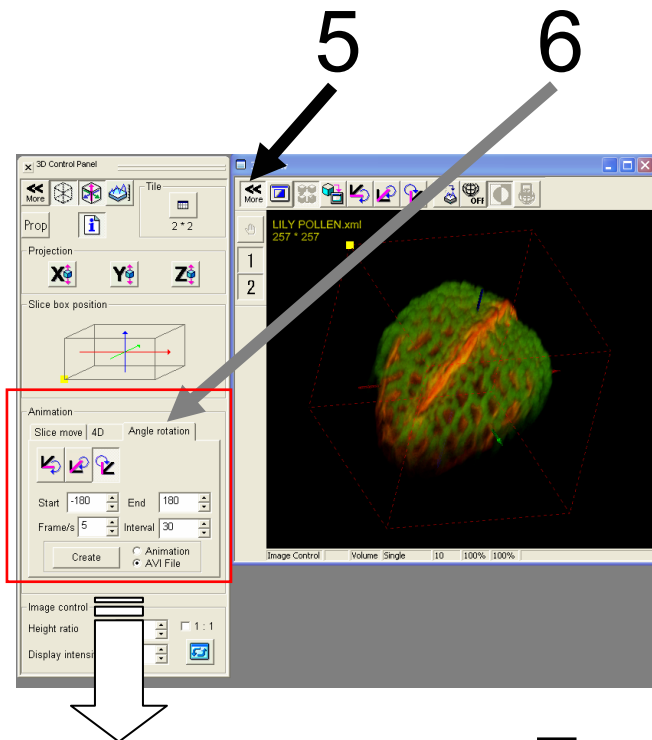
1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

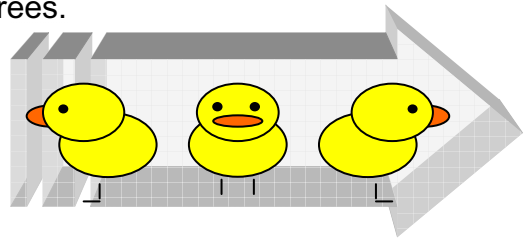
Image Analysis

(Rotating a Three-dimensional animation)



To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the  button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

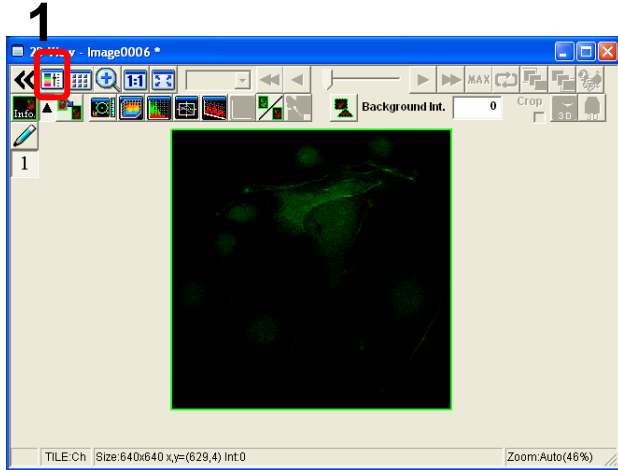
8. Enter the rotation angle.

Start = Angle to start rotation
End = Angle to stop rotation
Frame/s = Rotation speed
Interval = Degrees to be rotated at a time

9. Select AVI File and click on Create.

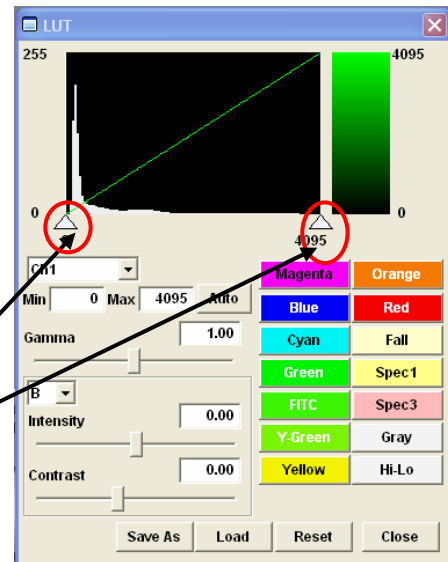
10. Enter a file name and click on Save.


2D Image Analysis (Edit the image color and contrast)



1. Click  "LUT" and then LUT table appears below,

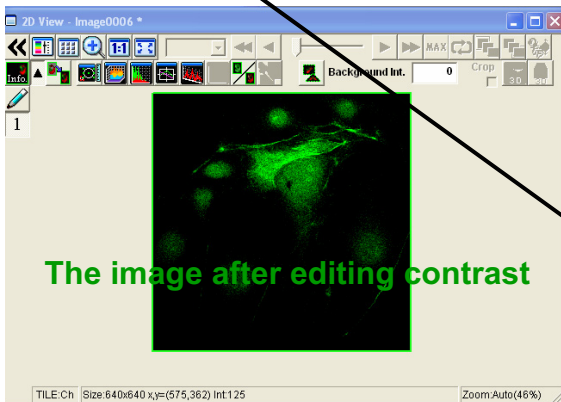
2



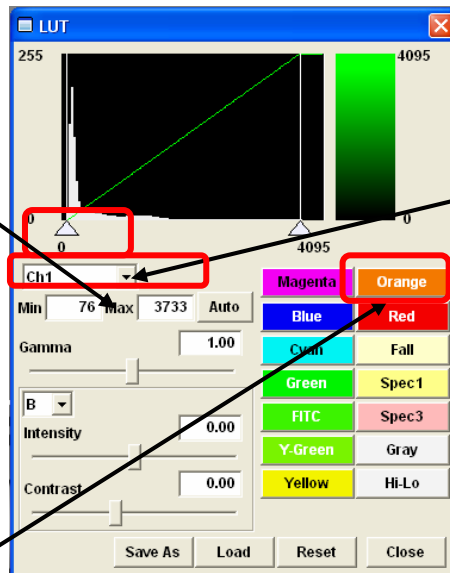
2. Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min" (Max4095, Min0)

3. Min and Max value are changed and contrast of image is edited.

* According to get Min value up , be able to reduce noise of the image.

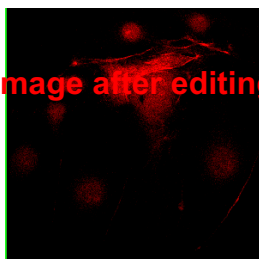


3



Edit each Ch

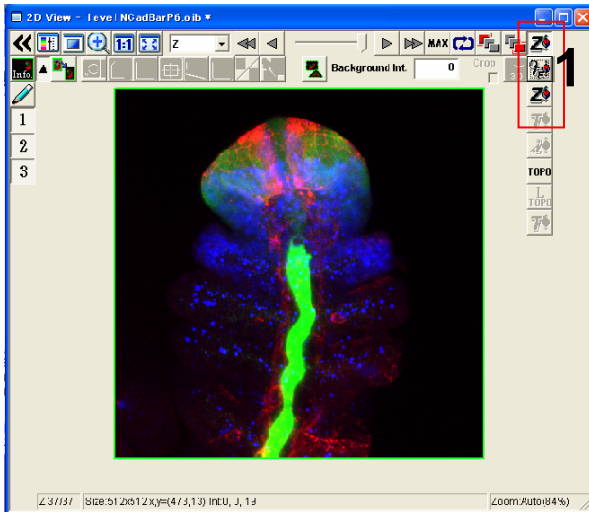
The image after editing color





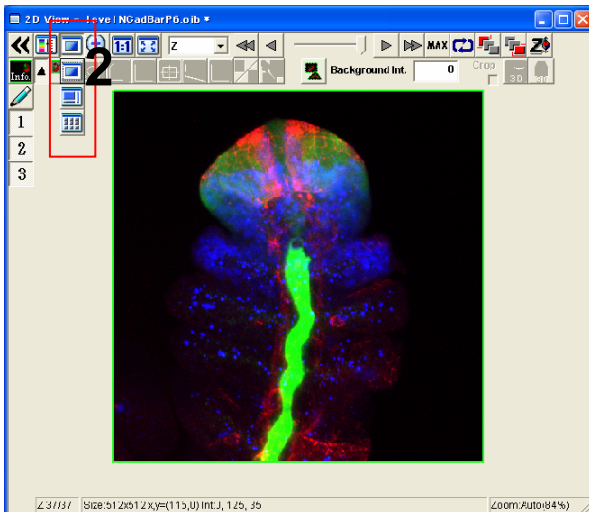
4. To click another color, be able to Edit a color.
Above example: Change Green to Red to click



Red

2D Image Analysis (the image of Z section)

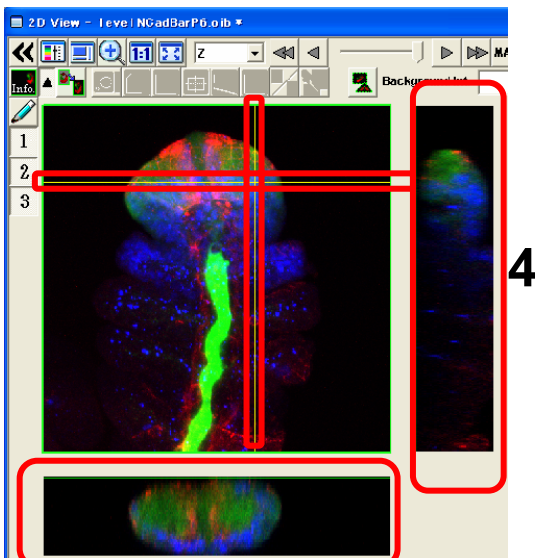


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select .

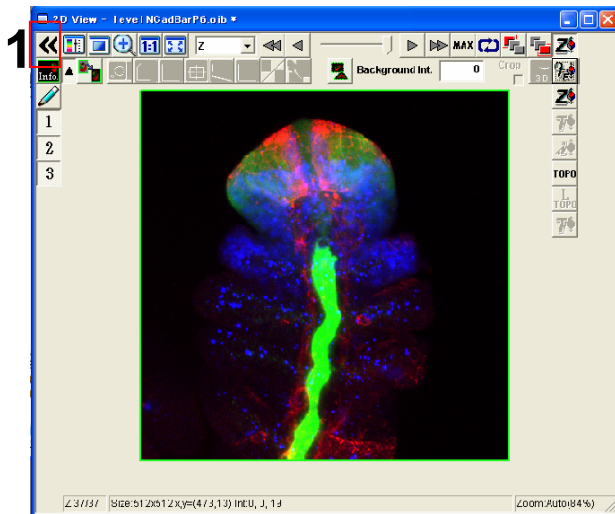
3. The images of Z section is shown on X axis and Y axis.
According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.




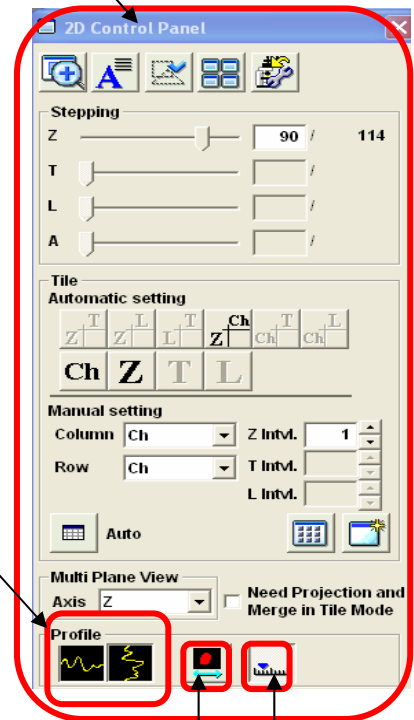
4. The image of Z section on Y axis.

5. The image of Z section on X axis.

2D Image Analysis (Intensity Profile of each Z sections)

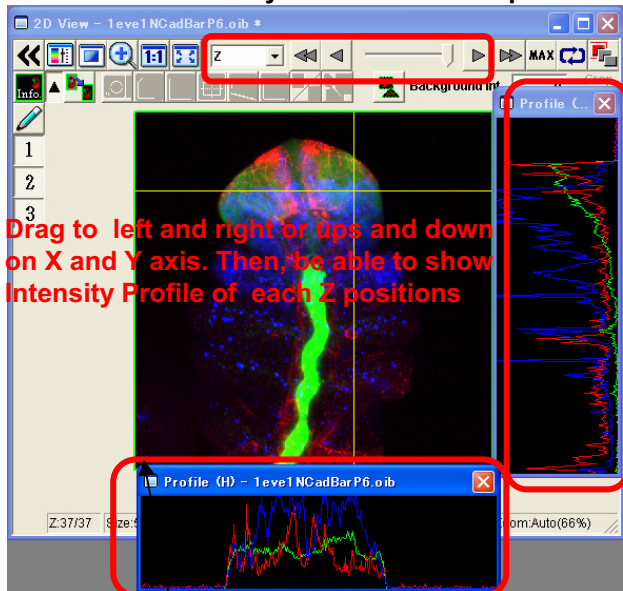


1. Click  and then 2D Control Panel is shown below,



2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.


To move to Z position ,be able to show Intensity Profile on each Z positions.



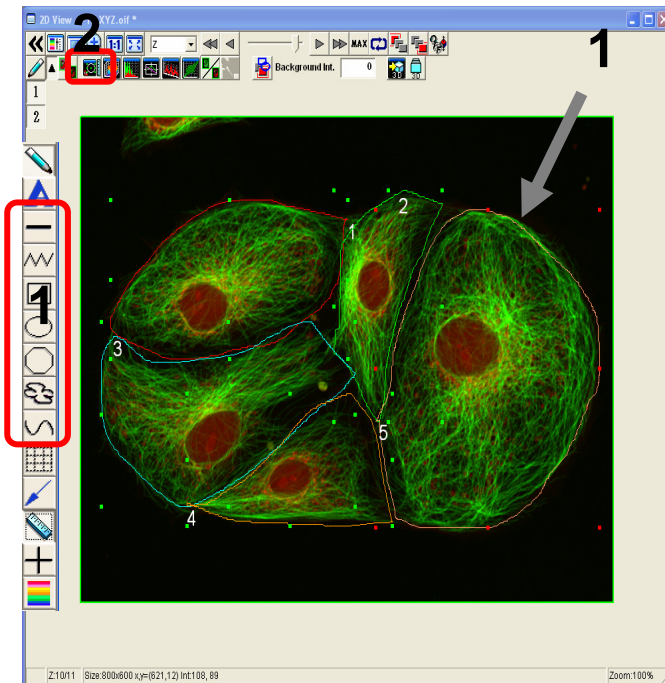
2

4 3


3. Click  to show Scale on Intensity Profile

4. According to click , be able to show as equal scale of Profile window as 2Dimage.

2D Image Analysis (Measure)



1. Enclose interesting regions by ROI

Line on interesting positions by ROI 

2. Click  "measure".

Region Measurement - PTK XYZ.tif Z:10 T:0 L:0 ROI:5

Image: PTK XYZ.tif Measure All ROIs

3. The information of ROI is calculated on Region Measurement.

Measure	ROI No. 5	Statistics	CHS1	CHS2
CenterX	150.780	Integration	121878548.000	54771708.000
CenterY	79.732	Average	1244.509	559.277
Area	6120.813	Max	4095.000	3227.000
Perimeter	213.258	Min	96.000	41.000
Range		Range	3999.000	3186.000
StdDev	41.309	StdDev	2175.309	1318.002
3StdDev	123.928	3StdDev	6525.927	3954.006

4. According to click "Measure All ROIs", then the information of all ROI is calculated on Region Measurement.

5. The information of all ROIs

ROI	CenterX [um]	CenterY [um]	Area [um^2]	Perimeter [um]	Integration CHS1	Average CHS1	Max CHS1	Min CHS1	Range CHS1	StdDev CHS1	3StdDev CHS1	Integration CHS2	Average CHS2	Max CHS2	Min CHS2	Range CHS2	StdDev CHS2	3StdDev CHS2
1	57.171	48.438	3129.625	241.490	5478264.000	1107.926	4095.000	95.000	4000.000	710.261	2130.783	2952481.000	658.076	3590.000	28.000	3562.000	522.518	1567.554
2	112.522	53.402	1470.188	194.764	9620457.000	1301.724	4095.000	97.000	3998.000	883.602	2650.807	7837013.000	758.280	3468.000	28.000	3440.000	561.877	1685.630
3	51.900	87.103	3274.688	273.215	2573667.000	1003.410	4095.000	94.000	4001.000	700.397	2101.192	9839166.000	569.504	3415.000	53.000	3362.000	443.623	1330.869
4	80.180	111.524	1732.438	211.246	4386227.000	879.766	3836.000	83.000	3753.000	657.656	1972.967	7880740.000	645.072	3380.000	25.000	3355.000	523.061	1569.183
5	150.780	79.732	6120.813	313.258	1878548.000	1244.509	4095.000	96.000	3999.000	725.103	2175.309	4771708.000	559.277	3227.000	41.000	3186.000	439.334	1318.002

Count: 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5

Average: 90.511 76.240 3145.550 246.795 5987432.600 1107.467 4043.200 93.000 3950.200 735.404 2286.212 9656221.600 638.042 3416.000 35.000 3381.000 498.083 1494.000

Max: 150.780 111.524 6120.813 313.258 1878548.000 1301.724 4095.000 97.000 4001.000 883.602 2650.807 4771708.000 758.280 3590.000 53.000 3562.000 561.877 1685.630

Min: 51.900 48.438 1470.188 194.764 4386227.000 879.766 3836.000 83.000 3753.000 657.656 1972.967 7837013.000 559.277 3227.000 25.000 3186.000 439.334 1318.002

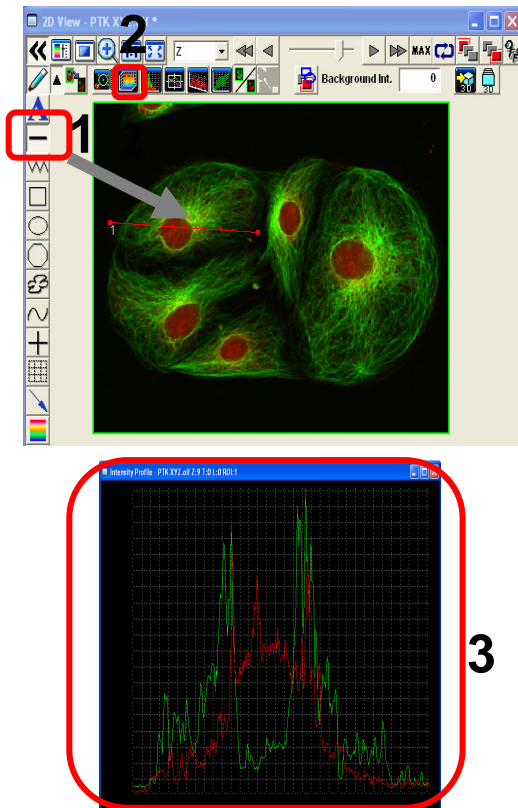
Range: 98.879 62.087 4650.625 118.495 7492321.000 421.958 259.000 14.000 248.000 225.947 677.840 5934695.000 199.002 363.000 28.000 376.000 122.543 367.000

StdDev: 41.309 25.569 1848.840 47.735 8699715.061 172.621 115.828 5.701 110.244 86.561 259.683 5124831.492 80.326 132.286 11.811 137.208 54.102 162.309

3StdDev: 123.928 76.707 5546.521 143.205 26099145.184 517.864 347.485 17.103 330.731 259.683 779.050 5374494.476 240.979 396.857 35.433 411.624 162.305 486.927


Save Histogram Clear Close

2D Image Analysis (Line Intensity Profile on the 2D image)



1. Line on the 2D image by ROI

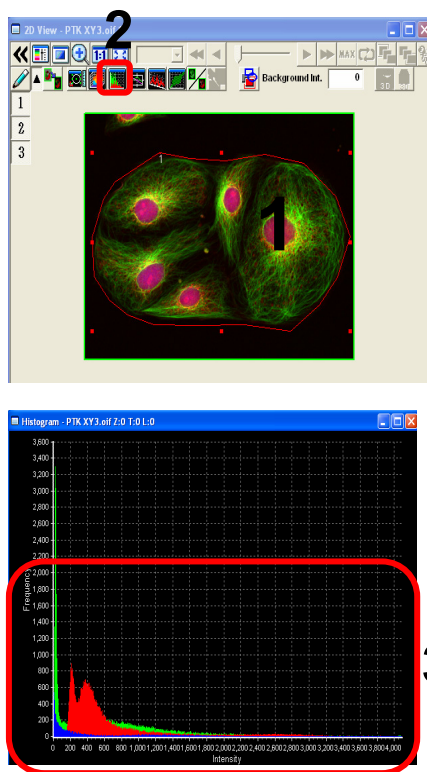


2. Click  "Intensity Profile"

3. "Intensity Profile" on the line is shown as intensity graph .

* State of colocalization between each Chs is figured out apart from intensity .

2D Image Analysis (Histogram)

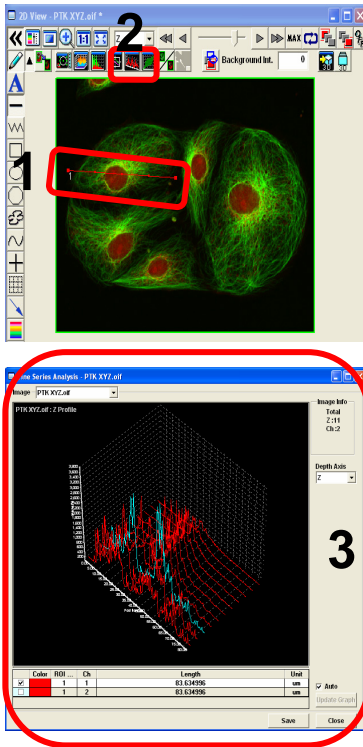


1. Enclose the interested region by ROI.


2. Click  "Histogram"

3. "Histogram" window is shown as a graph, frequency of intensity of each pixels is plotted on the region enclosed by ROI.

2D Image Analysis (Line Series Analysis)

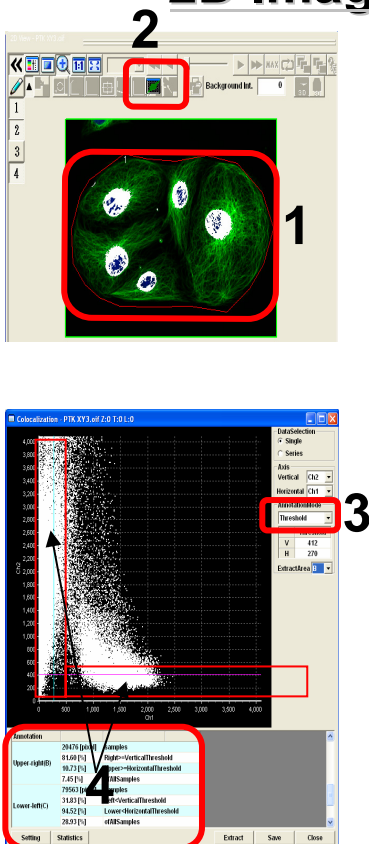


1. Line on the interesting region.

2. Click  “Line Series Analysis”

3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



1. Enclose an interesting area by ROI.

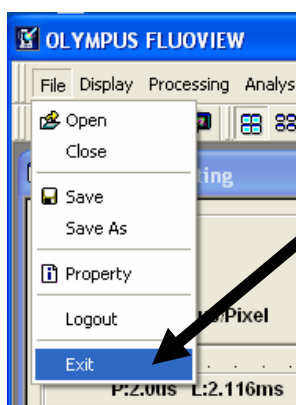
2. Click 

3. Select  **Threshold** from **Annotation Mode**.

4. According to move Thresholds of X,Y axis to right and left ,ups and down (**Enclose red color X,Y axis**), Co-localization result between 2ch is changed .

Information of Co-localization is listed under the scatter plot.

Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.

(2) On the Shut Down Window, select Shut Down and click on OK.

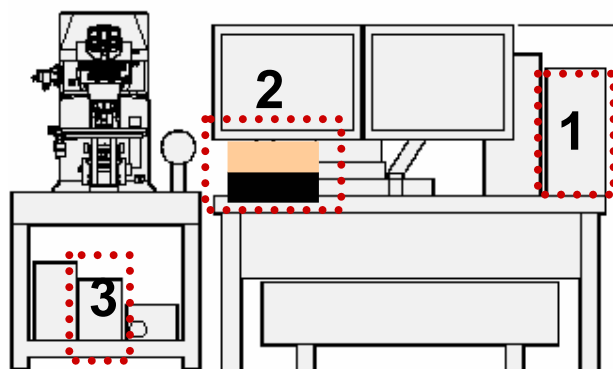
3. Turn the laser OFF.
(Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

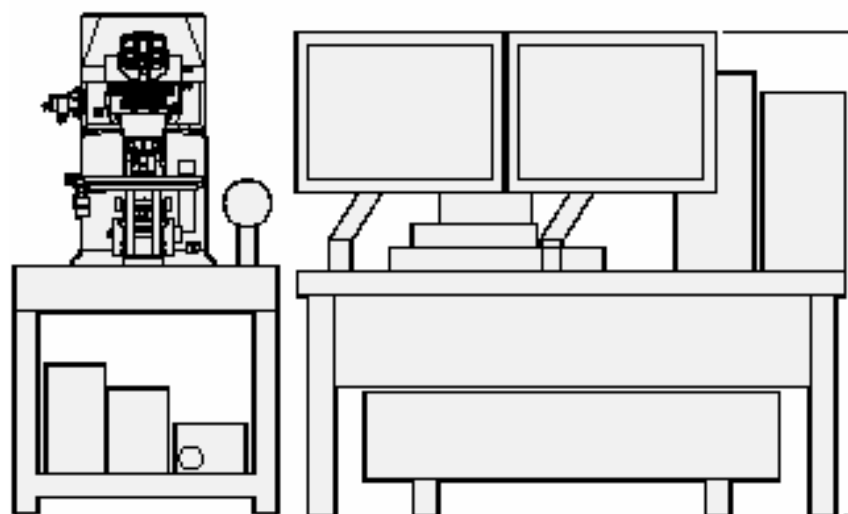
3-3. HeNe (G) (543 nm) OFF

4. Turn the mercury burner power OFF.





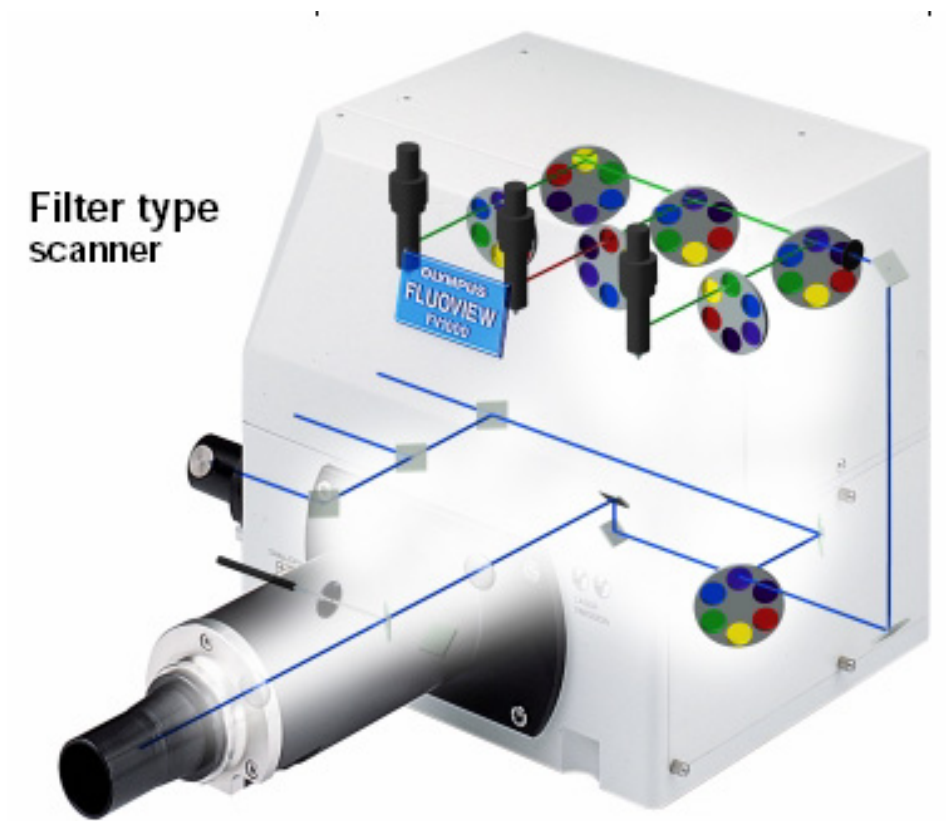
Laser Confocal Scanning Microscope FV1000D Filter Type (Upright Microscope BX61) Operation Manual



Contents

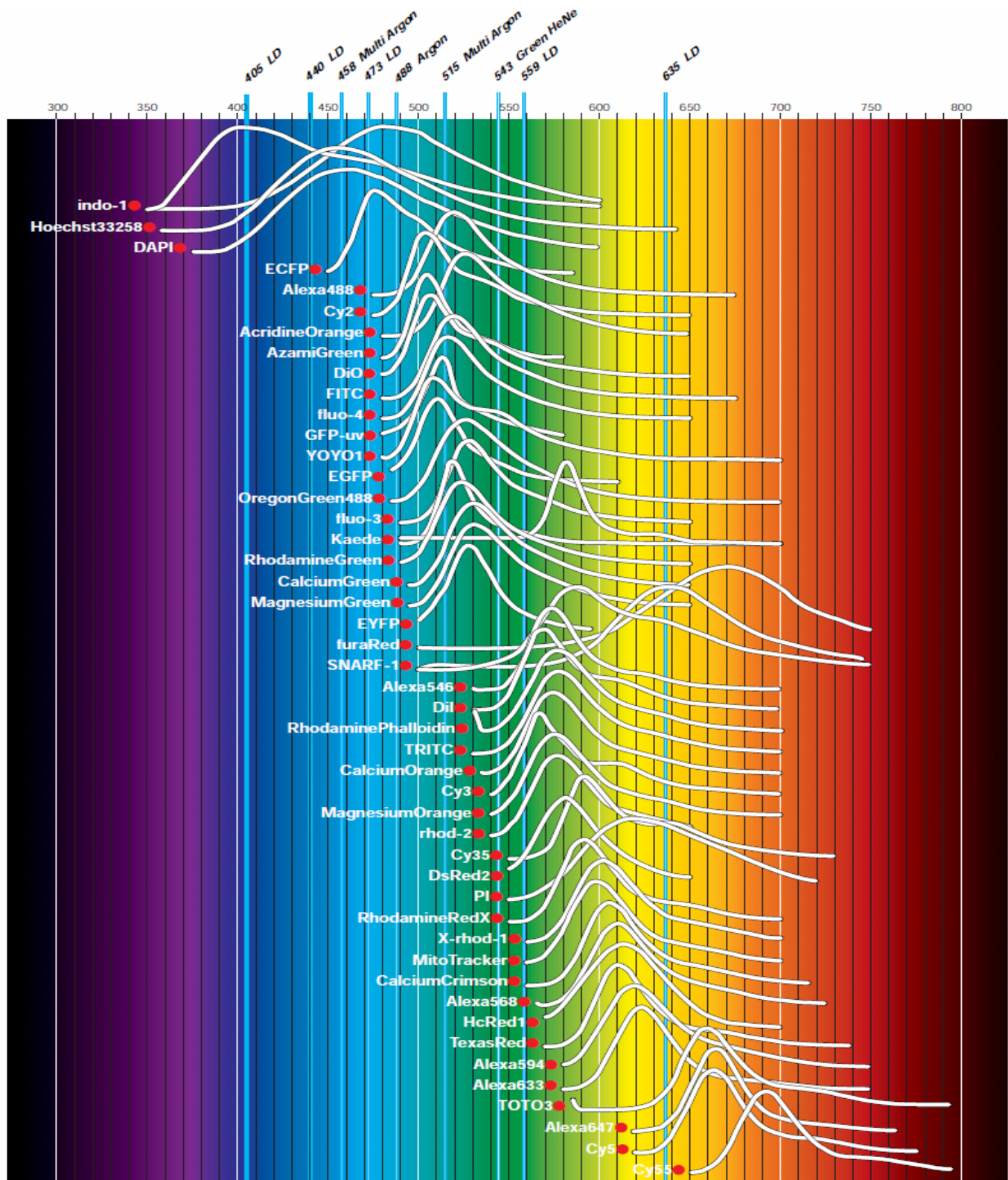
System introduction	3
FV1000D Laser DyeList	4
System Preparation	5
Visible Observation	
Observation of Fluorescence image	6
Observation of Differential Interference Contrast Images	7
Image Acquisition	
Overview of Operation Panel for Image Acquisition	8
Single Stain on XY Image	9-11
Complement of adjusting the image	12-13
Double Stain on XY Image	14-15
Sequential scan Line Sequential	16
Double Stain on XYZ image	17-18
Four Stain on XY Image	19-21
Single Stain + DIC on XY Image	22-23
Merge the image between fluorescent XY image and DIC image	24
Reload the image conditions	25
Image Analysis	
Overview of the 2D Operation Panel / Opening a file	26
Making 2D Z projection file Images	27
Saving a Z section image as 2D image	28
Inserting the Scale Bar	29
Rotating a Three-dimensional Image	30
Saving an Image	31
Saving Rotating a Three-dimensional animation file	32
2D Image Analysis	
Edit the image color and contrast	33
The image of Z section	34
Intensity Profile of each Z sections	35
Measure	36
Line Intensity Profile on the 2D image / Histogram	37
Line Series Analysis / Co-localization	38
Closing the System	39

Filter Type Main Scanner

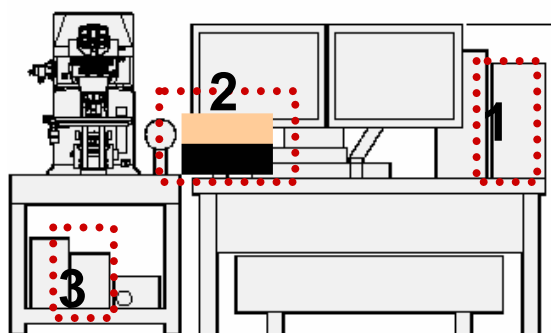


Dye List (FV1000D Lasers are available below)

LD405nm LD440nm LD473nm LD559nm LD635nm
Ar458nm Ar488nm Ar515nm
HeNe(G)543nm



System Preparation



1. Turn the computer ON.
[In case of equipped concentrated power supply, power on it first]

2. Turn the laser ON
(Turning the key switch)
2-1. LD559nm ON
2-2. Multi Ar 458nm 488nm 515nm
2-2. HeNe(G)(643nm) ON

3. Turn the mercury burner ON for Fluorescence observation.

4. Log on Windows

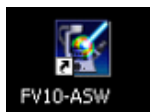
Enter Password, Customer name is below

User name: Administrator

Password : fluoview



Wait for a moment until the software is started



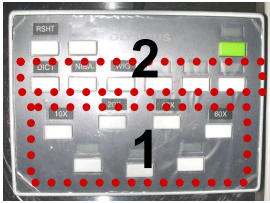
5. Double click this icon to log on to ASW

User name: Administrator

Password : Administrator

Visual Observation under the Microscope

■■ Observation of Fluorescence Image ■■



Hand switch



1. Select an objective lens
by using the hand switch

2. Select florescent filter cube

MEMO

Fluorescence filter

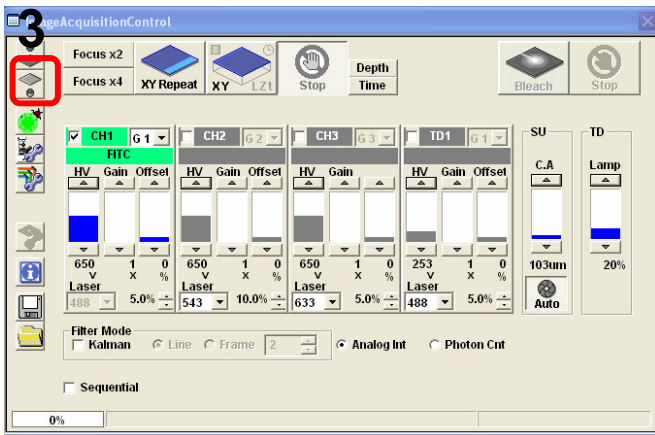
NIBA: Blue Excitation / Green Fluorescence
(Ex.:FITC,EGFP)

WIG: Green Excitation / Red Fluorescence
(Ex.:Rhodamine, DsRed)

3.



Click the button
on the Fluoview software



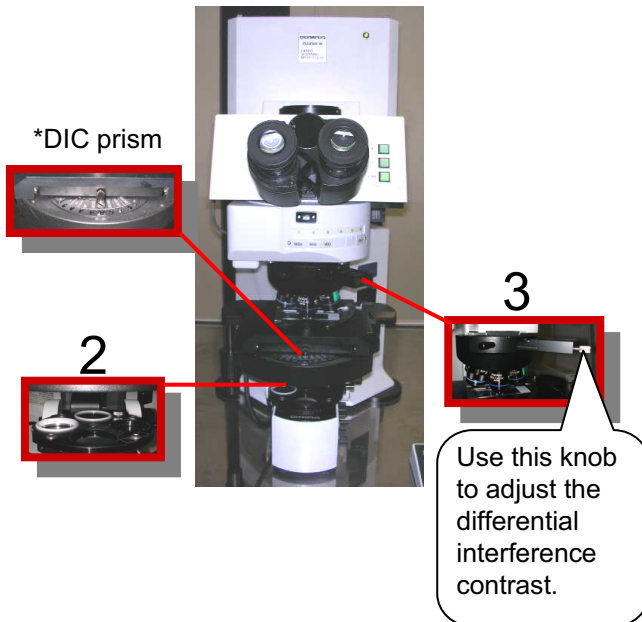
4. Focus to the specimen

Visual Observation under the Microscope

■■ Observation of Differential Interference Contrast Images ■■



Hand switch

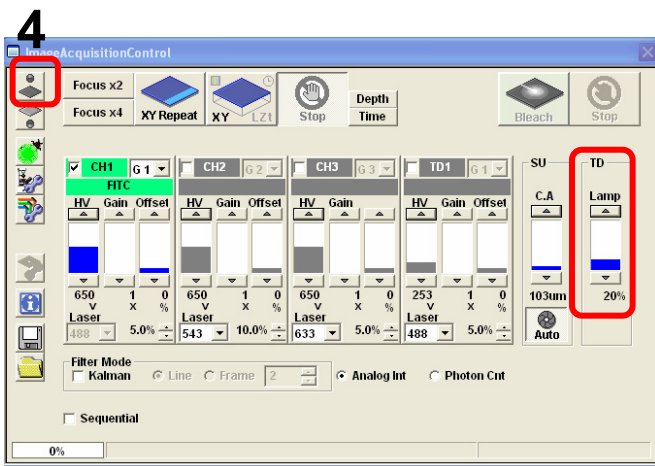


1. Select the Objective Lens

2. Insert the Polarizing Plate in the Light Pass

3. Insert the DIC prism slider in the light pass

4.  Click the button on Fluoview software



5. Focus to the specimen

Overview of Operation Panel for Image Acquisition

The image displays three screenshots of the image acquisition software interface, with various components labeled and described.

AcquisitionSetting

- Scan mode
- Scan speed
- Number of pixels
- Zoom & Pan
- Laser output adjustment
- Objective lens
- Focus
- Time Interval & Time Number (for acquisition of XYT or XT image)

ImageAcquisitionControl

- Transmitted light observation (visual observation)
- Fluorescence observation (visual observation)
- DyeApply
- Optical path diagram
- TwinScanner setting
- Save acquisition conditions
- Load acquisition conditions
- Scan buttons
- Select XYZ, XYT or XYL
- Adjustment of each channel
- Confocal aperture
- Light intensity adjustment for halogen bulb
- Kalman

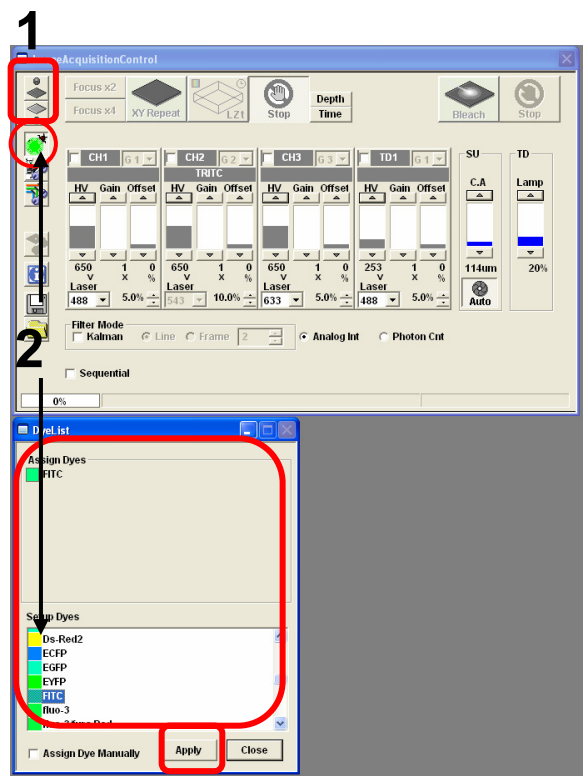
Image View



- Image display window
- Image file thumbnail
- Display of files in the memory

Image Acquisition (Single Stain on XY Image)

■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Single stain of green fluorescence dye (FITC)



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

3. Click on the Apply button.

(The DyeList panel can be closed by using the Close button.)

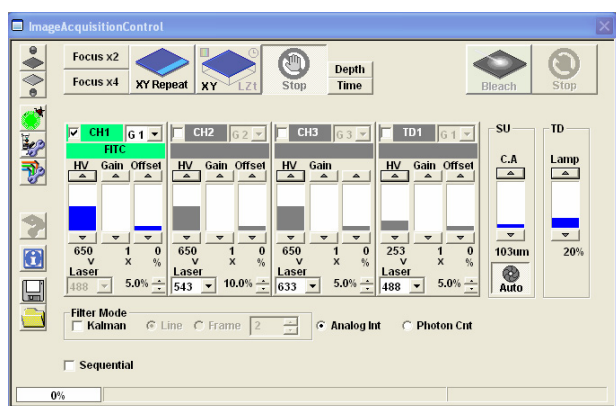
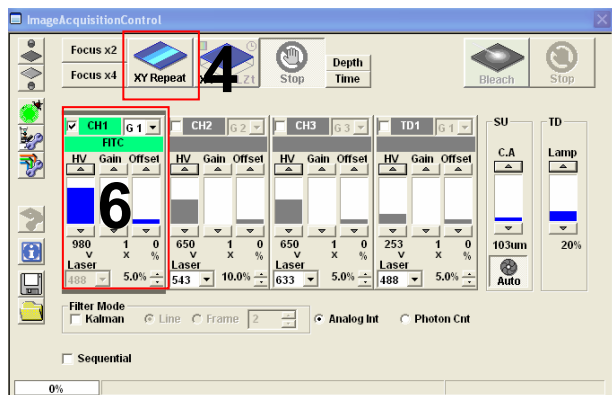


Image Acquisition (Single Stain on XY Image)



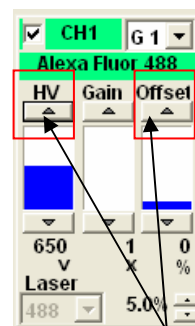
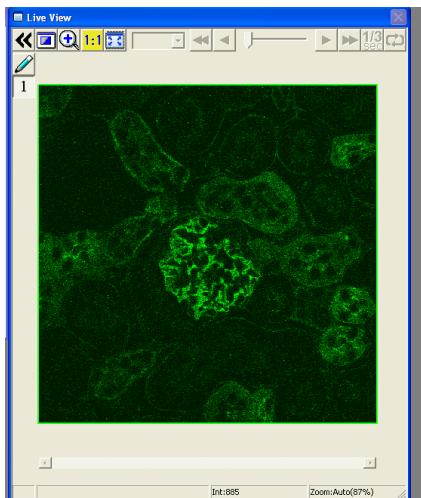
4. Press XY Repeat button click to get image



: Continuous scan mode

5. Focus to the specimen

6. Adjust the green (FITC) image.



· Adjust sensitivity of **HV** and reduce noise by **offset**

7. Press keyboard **Ctrl + H key**

Optimized PMT adjustment brightness intensity 2 color between white and black,

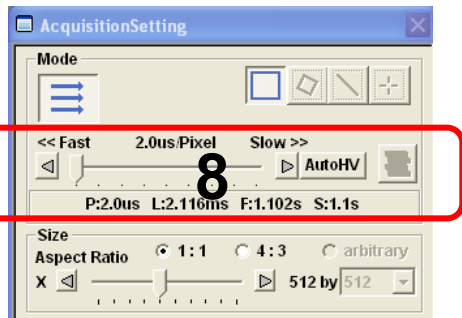
Maximum intensity is 4095(12bit) if intensity is over 4095, color is changed to red (saturation)

* Basically, **Gain value is 1**



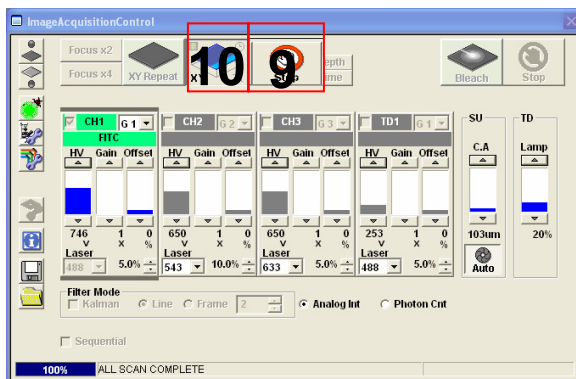
7


Image Acquisition (Single Stain on XY Image)




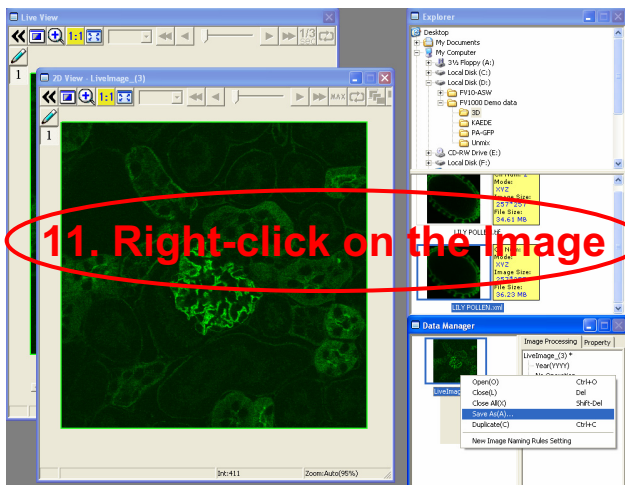
8. Select AutoHV and then select ScanSpeed.

*As the scan speed becomes slower, noise can be removed while maintaining the current brightness.



9.  Press the Stop button to stop scanning.

10.  Click on XY, and "2D View-LiveImage(x)" is displayed on the window bar for the image that has been acquired.



11. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.)

Save the image as TIFF, BMP, JPEG format Select "Export" and chose the format TIFF, BMP, JPEG.

■Memo■

File formats specifically for the FV10-ASW

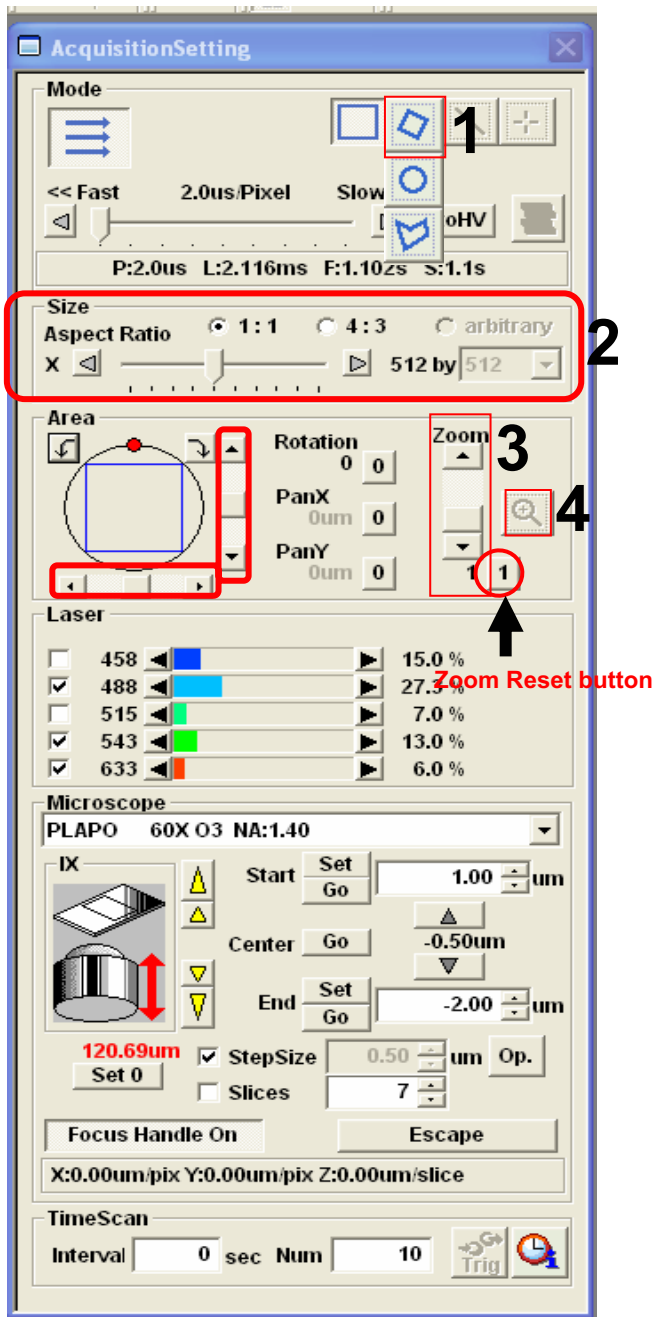
OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

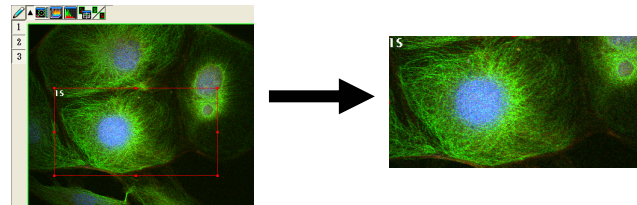
OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Complement of adjusting the image



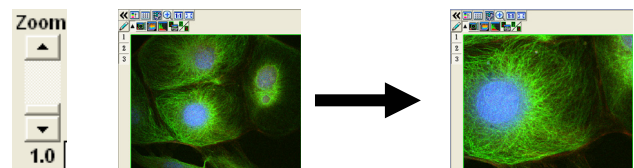
1. Click "Clip scan" button , and enclose a interesting region's image on the whole image.




2. pixel setting
* The standard pixel is 512 x 512

3. Zoom Setting

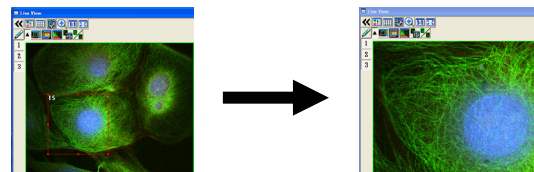
Press "XY Repeat" to scan and set zoom value.



Above image is zoomed From 1x to 2
* Scan speed and pixel resolution remain even zoom value is changed

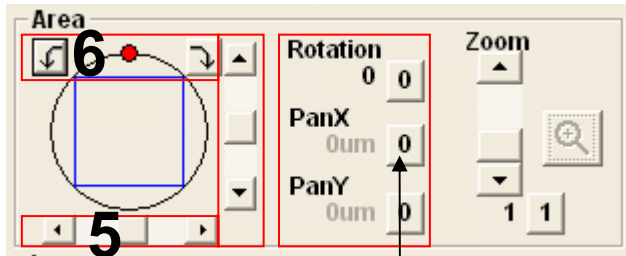
4. Click  Zoom scan, and be able to enclose an interesting region's on the whole image

Press XYRepeat to scan after enclosing the area

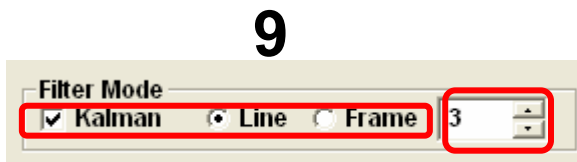
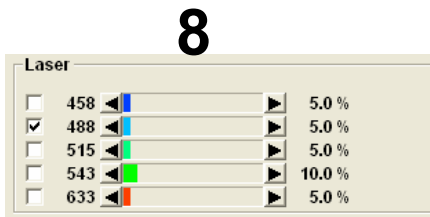


* Scan speed and pixel resolution remain even zoom value is changed

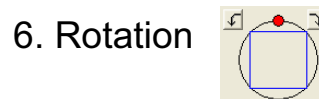
Complement of adjusting the image



PanX,Y and Rotation reset button



Be able to move the field of view to set Pan X,Y without stage action



Be able to rotate the whole image.



Click "Auto" button to acquire Optimized Confocal aperture
Confocal aperture . . . change confocal aperture to larger diameter for dim fluorescence image then, be able to get the more bright image. But Z axis resolution gets worse.

Laser Intensity . . . More Laser intensity is increase , more bright image is .

* More increase laser intensity is , more discoloration image is .

Kalman accumulation . . . Image acquisition is repeated to the specified number of times to provide an averaged image. Consequently, noise is averaged and roughness on the whole image is reduced.

Advantage: The speed of each scan is fast.

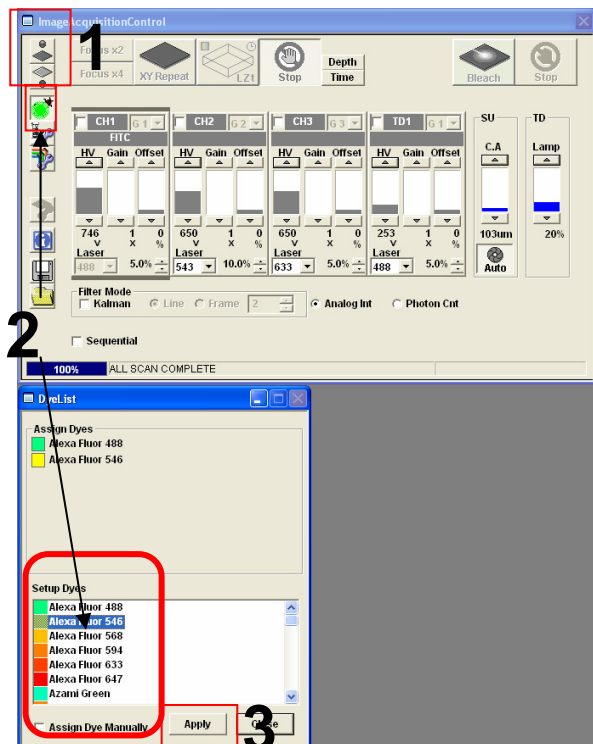
Disadvantage: Some blur occurs due to averaging of images.


Image Acquisition (Double Stain on XY Image)


■ Acquisition of a single image (XY plane) (fluorescence image only) ■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Simultaneous scan



1. Click on the FV10-ASW software button  to close the fluorescence lamp shutter.

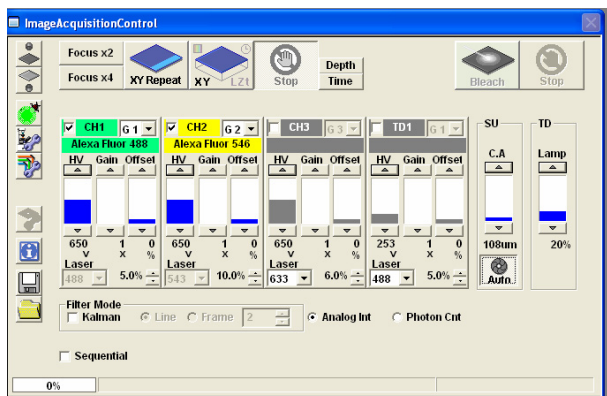
Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

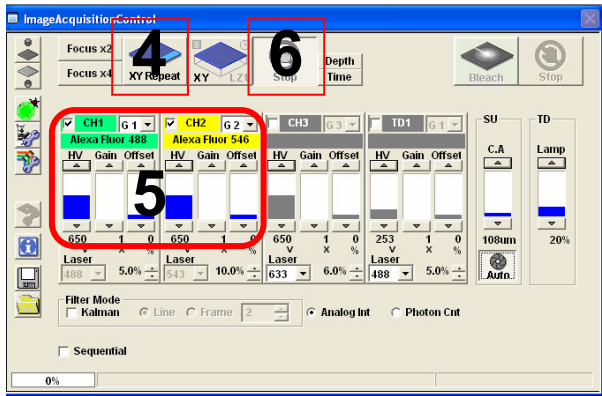
3. Click “Apply” button.

(The DyeList panel can be closed by using the Close button.)



Display after DyeApply is carried out

Image Acquisition (Double Stain on XY Image)



4. Press the XY Repeat button to start scanning.

5. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.

(The image adjustment is outlined below.
For more information, refer to Appendix 1.)

6. Press the Stop button to stop scanning and press XY repeat to acquire the image. (Refer to ■Memo■.)

■Memo■

Scan buttons



: Continuous scan

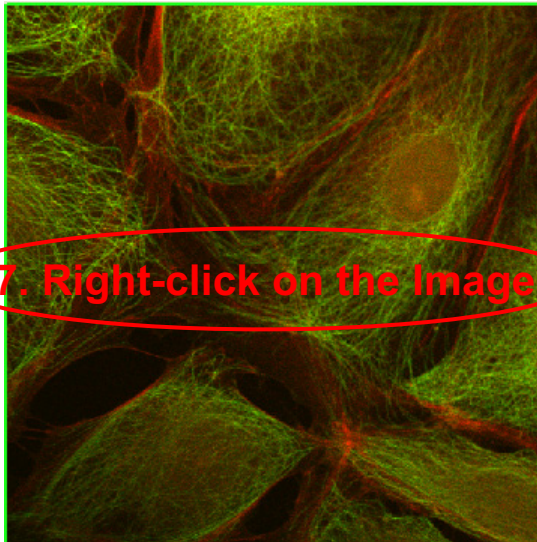


: Stop scan



: Rough scan (Line skipped)

7. Right-click on the Image



7. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)

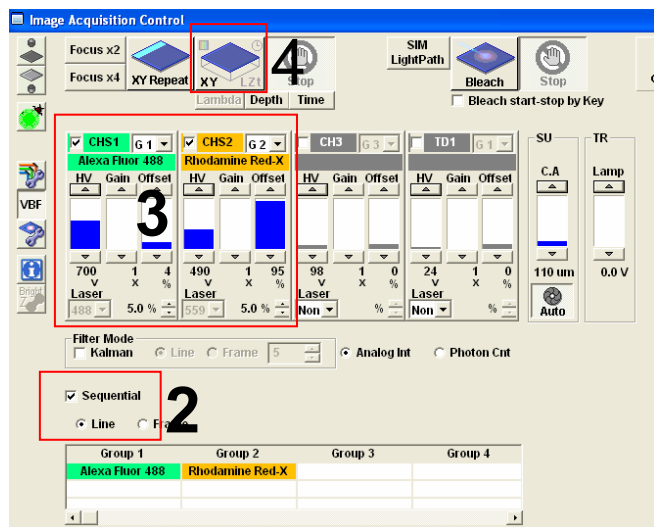
Image Acquisition

(Double Stain on XY Image)

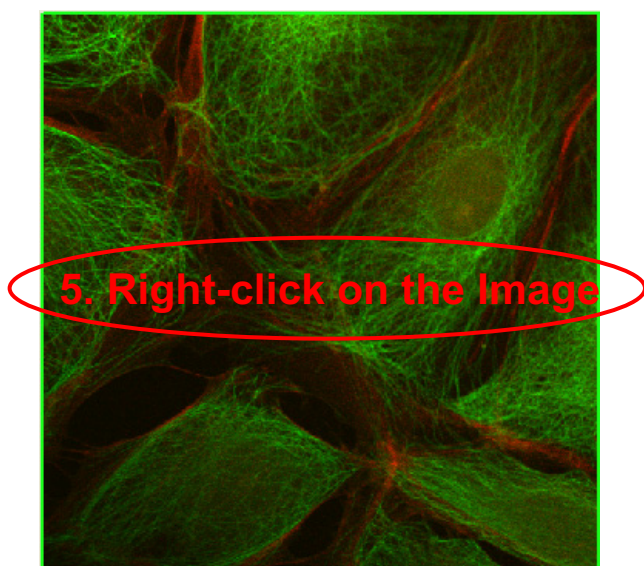
■■ Acquisition of a single image (XY plane) (fluorescence image only) ■■

Sample: Double stain of green fluorescence dye (Alexa 488)
and red fluorescence dye (Alexa 546)

Sequential scan (Line Sequential is introduced here.)



1. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.
2. Check Sequential and select Line.
3. Adjust the green (Alexa Fluor 488) image and the red (Alexa Fluor 546) image.
4. Press the XY button to acquire an image.
5. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.) The image is acquired.



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

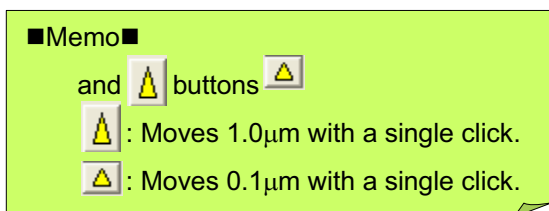
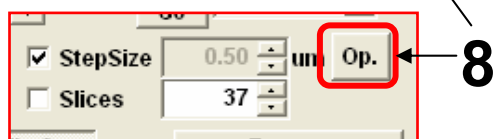
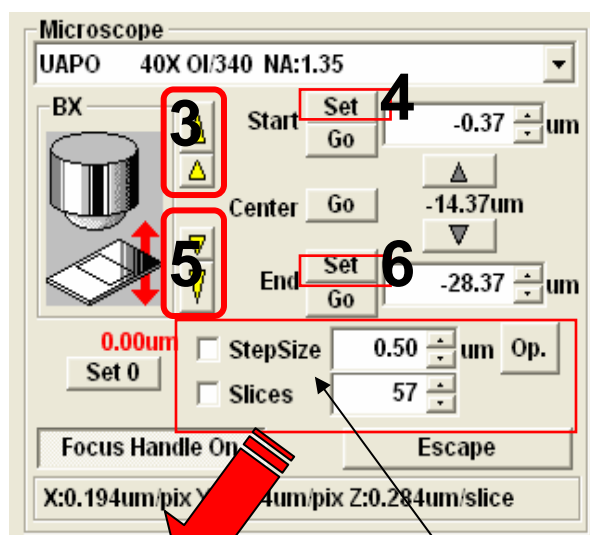
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Double Stain on XYZ Image)

■ Acquisition of 3D images (XYZ) (fluorescence image only) ■

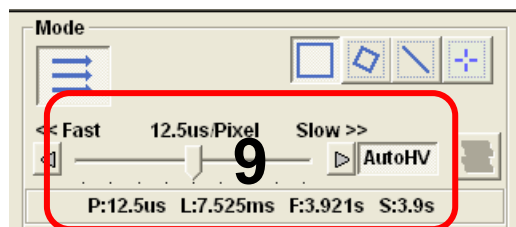
Sample: Double stain of green fluorescence dye (FITC)
and red fluorescence dye (Rhodamine)

This is the procedure to acquire images
through Line Sequential scanning.



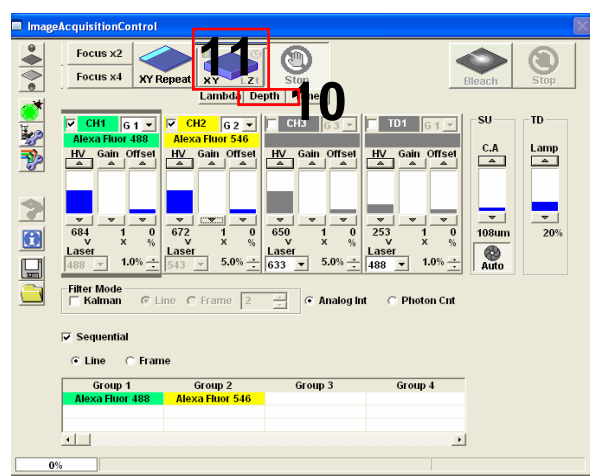
1. Take steps 1 to 7 described on pages 13 and 14.
2. Press the XY Repeat button to start scanning.
3. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
4. When the sample upper limit is displayed on the image, accept it using the Set button.
5. Click on the and buttons to shift the focal point. (Refer to ■Memo■.)
6. When the sample lower limit is displayed on the image, accept it using the Set button.
7. Press the Stop button to stop scanning.
8. Enter StepSize, Slice (the recommended value can be referred to by using the Op button), and check the check box

Image Acquisition (Double Stain on XYZ Image)



9. Select AutoHV and then select ScanSpeed.

10. Select Depth.



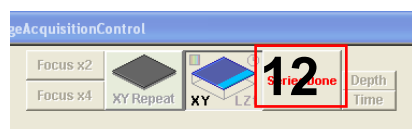
11. Press the XYZ button to acquire an image.

12. Click on “SeriesDone”, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.

13. Saving the image:

Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.

(Save as Type “oib” or “oif” file format specifically for the FV10-ASW software.)



■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

13. Right-click on the Image

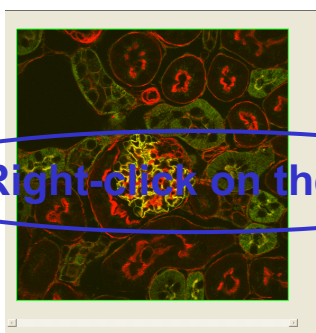
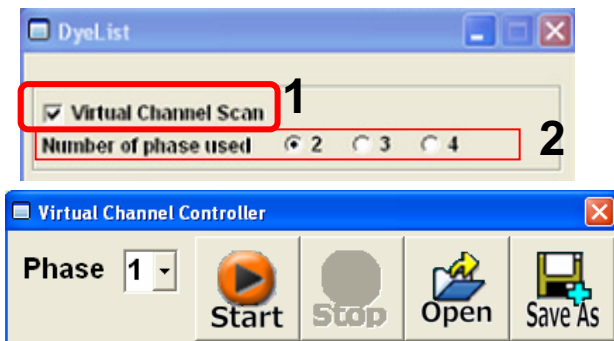


Image Acquisition (Four Stain on XY Image)

■ Acquisition of 4 stain images (XY)
(fluorescence image only) ■

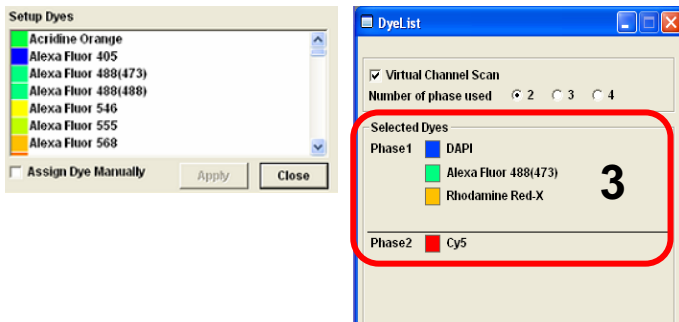
Sample: Four stain of Blue fluorescence dye (DAPI), green fluorescence dye (Alexa488) and red fluorescence dye (Rhodamine), far-red fluorescence dye (Cy5)

This is the procedure to acquire images through Virtual Channel scan

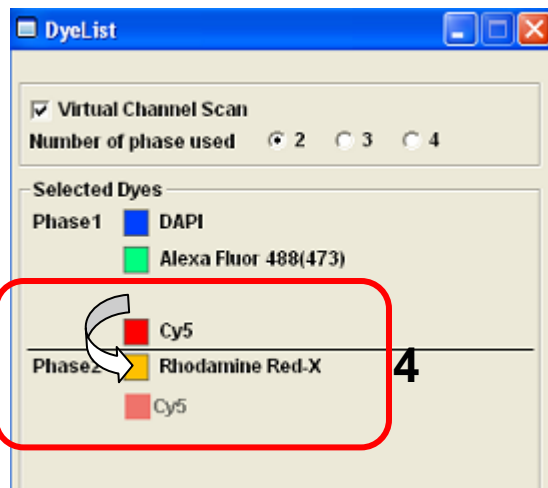


1. ☒ Virtual Channel Scan Select Virtual channel Scan on the DyeList, and then **“Virtual Channel Controller”** is automatically turned on.

2. Select a number of Virtual Channel from **“Number of phase used”**.



3. Select 4dyes from DyeList 4th dye is registered in **“the Phase 2”**.



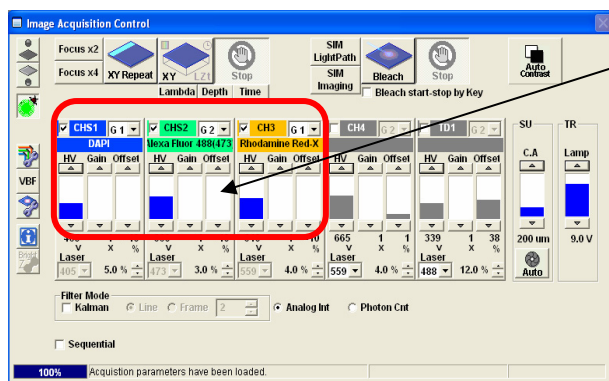
* RodaminRed is able to be registered on **“Phase2”** to drag .

Image Acquisition (Four Stain on XY Image)



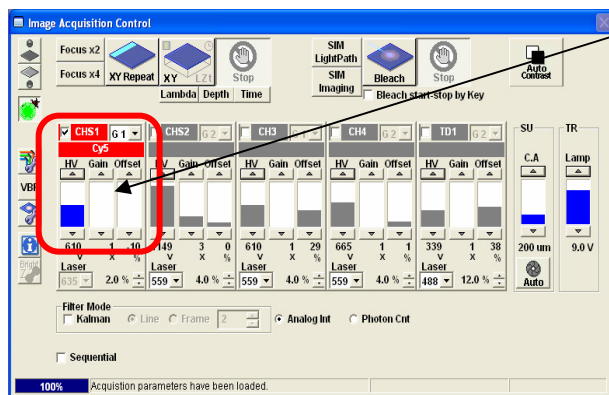
4. Select “Phase1”, “DAPI”, Alexa488, RhodaminRed are registered on ImageAquisitionControl.

* Slit and Filter, DM are automatically set for “DAPI”, “Alexa488”, “PhodaminRed”



5. Select “Phase2”, Cy5 is registered on ImageAquisitionControl

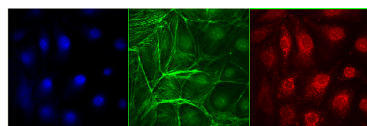
* Slit and Filter, DM are automatically set for “Cy5”



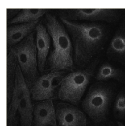
Adjust the image at each phases


“Phase1”

“Phase2”



6




6. Adjust the image to click  “XY Repeat” at each phases

* If acquire XYZ image, be able to decide upper limit and bottom limit , slices, step size of Z axis at both phases.

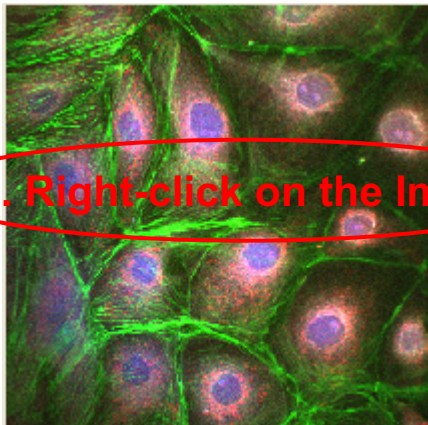
Image Acquisition (Four Stain on XY Image)



7

7. Click  on Virtual Channel Controller to acquire the image.

* Be able to start at each Phase.



8. Right-click on the Image

8. Saving the image: Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as Type "oib" or "oif" file format specifically for the FV10-ASW software.

■Memo■

File formats specifically for the FV10-ASW

OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

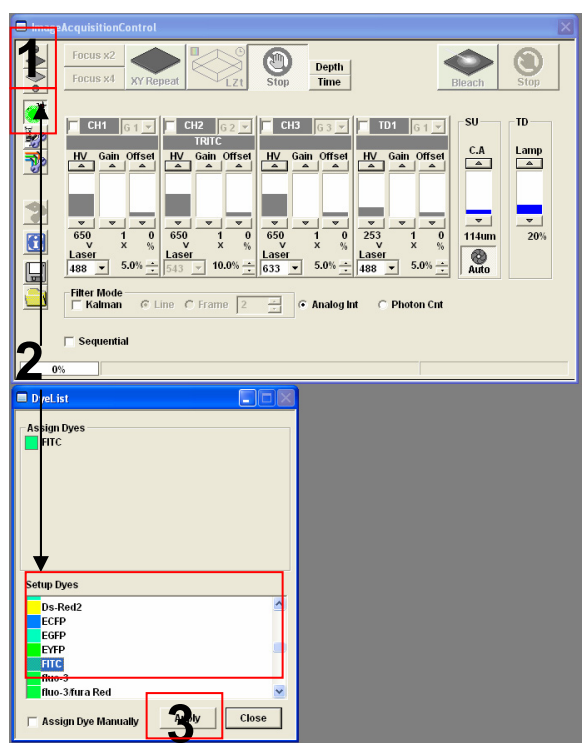
OIB format:



Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Acquisition (Single Stain + DIC on XY Image)

■■ Acquisition of a single image (XY plane)
(fluorescence image and differential interference contrast image) ■■

Sample: Green fluorescence dye (FITC) and differential interference contrast image

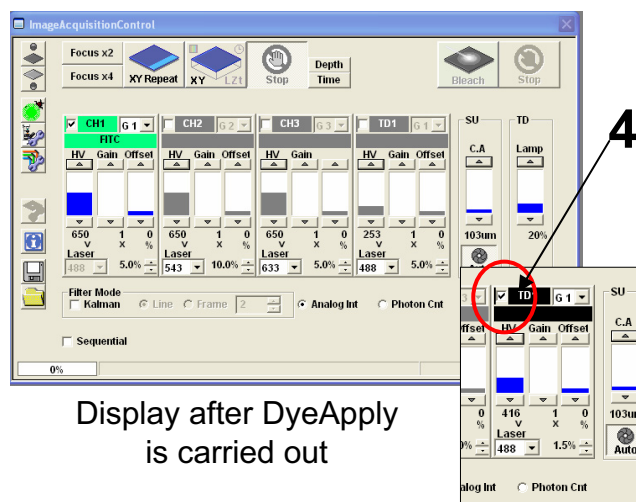


1. Click on the FV10-ASW software  button to close the fluorescence lamp shutter. Alternatively, click on the  button to close the halogen bulb shutter.

2. Click on the DyeList button. On the DyeList panel, double-click on a fluorescence reagent to be used for observation.

* To cancel the selection and select a different reagent, double-click on the fluorescence dye listed on the Assign Dyes window and take step 2 again.

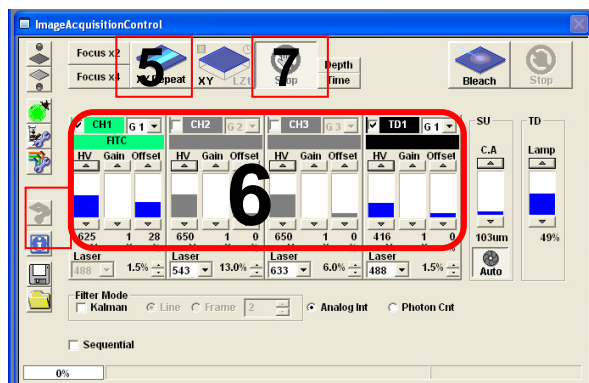
3. Click on the Apply button.
(The DyeList panel can be closed by using the Close button.)



Display after DyeApply
is carried out

4. Check TD1.

Image Acquisition (Single Stain + DIC on XY Image)



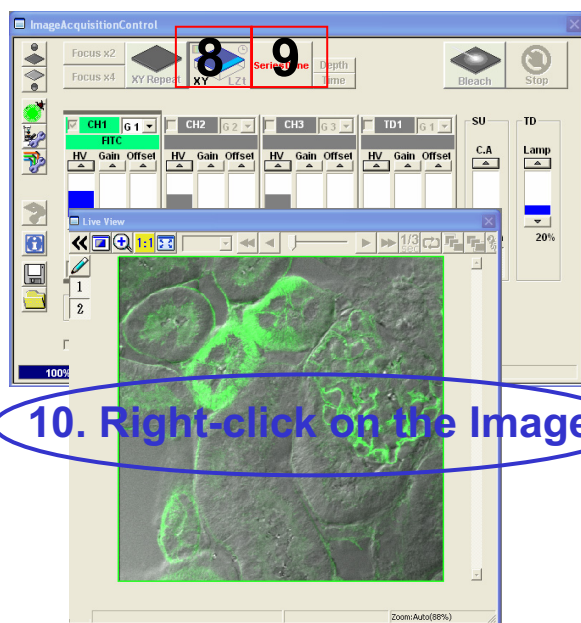
5. Press the “XY Repeat” button to start scanning.

6. Adjust the green (FITC) image and the differential interference contrast image.

7. Press the “Stop button” to stop scanning.

8. Press the “XY button” to acquire an image.

9. Click on “SeriesDone”, and “2D View-LiveImage(x)” is displayed on the window bar for the image that has been acquired.



10. Right-click on the Image

10. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image.
(Save as Type “oib” or “oif” file format specifically for the FV10-ASW)

■Memo■

File formats specifically for the FV10-ASW

OIF format:

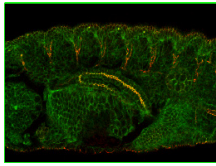
Creates “a folder that contains an image (16-bit TIFF)” and “an accessory file,” which cannot be opened separately from each other.

OIB format:

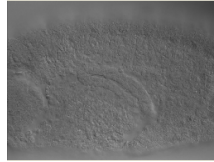
Creates the OIF format files in a single file, which is convenient for migration and other operations.

Merge the images between fluorescent XY image and DIC image

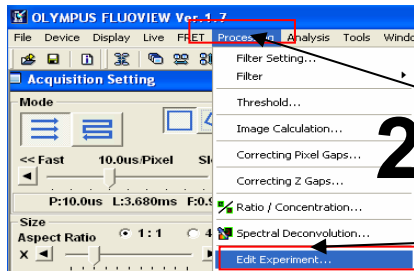
Edit different each files to the same file. This is available for making merge image
Between fluorescent image and focused DIC image.



1

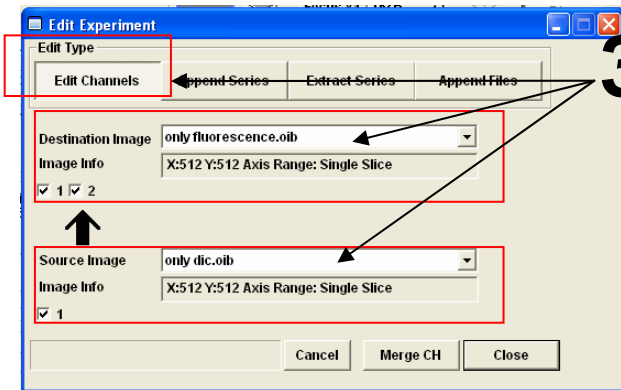


1. Open fluorescent image and
DIC image.



2

2. Select
Edit experiment from **Processing**

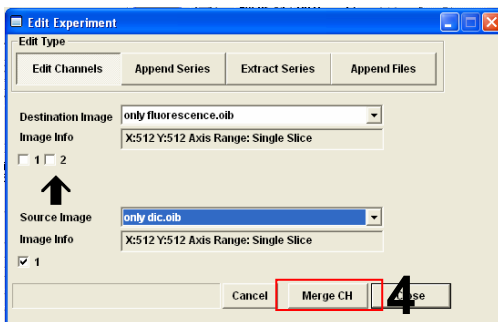


3

3. Click **Edit Channels** , and select
fluorescent image file at
Destination Image , select DIC
image at **Source Image**.

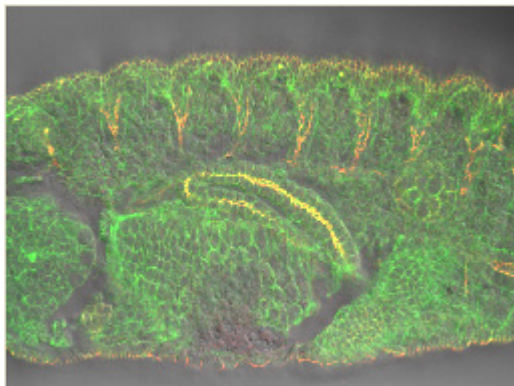
Image Info

* Check Image Info ☒ 1 ☒ 2 to make the.
merge file. If all channels are checked ,all
channels are reflected in the new merged file.



4

4. Click **Merge CH** , and then
the fluorescent image and the DIC
image are merged as the new file .

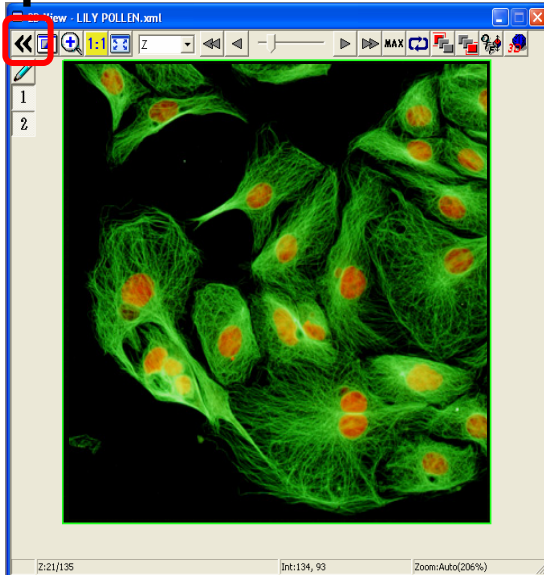


5

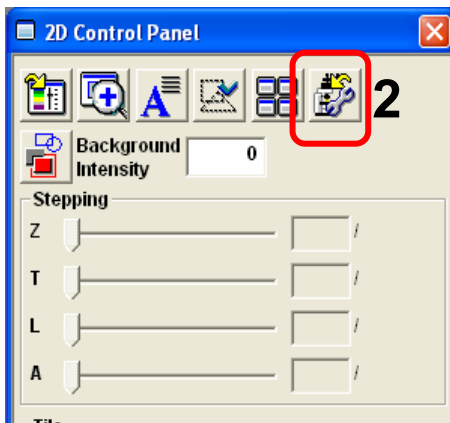
5. Merged image between the
fluorescent image and the DIC
image.

Reload the image conditions

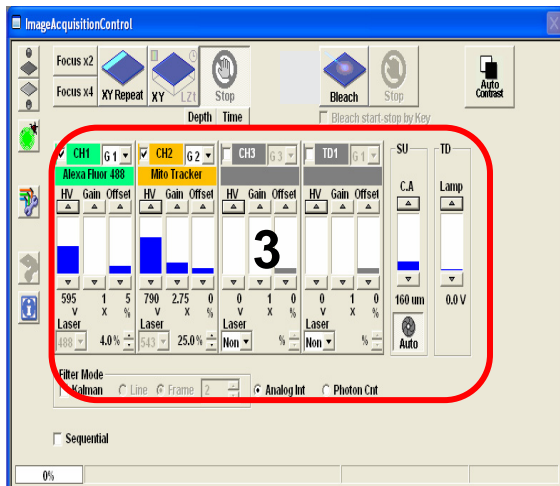
1



1. Open the file and click



2. Click



3. The conditions (HV,Offset, CA and so on) are reloaded .

Overview of the 2D Operation Panel

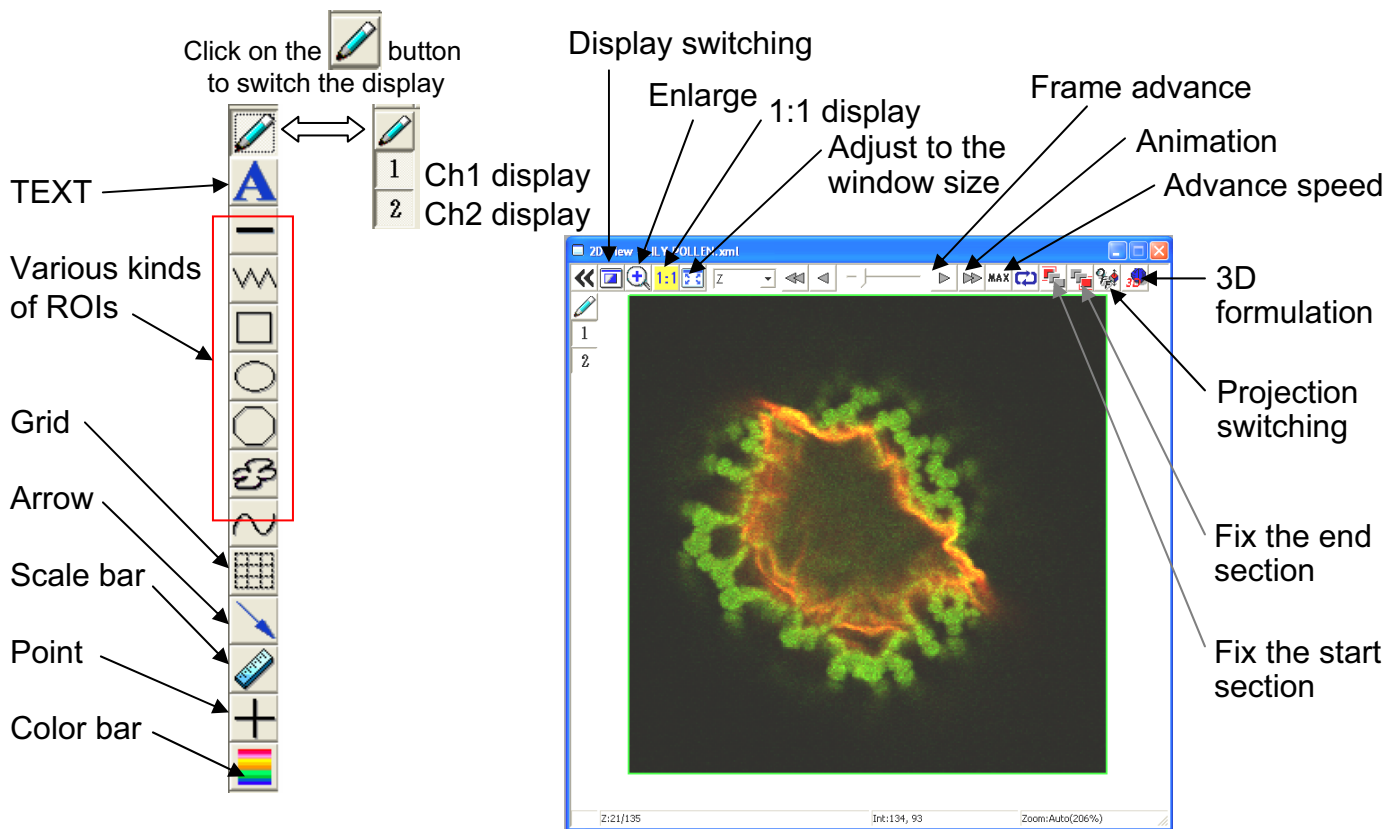
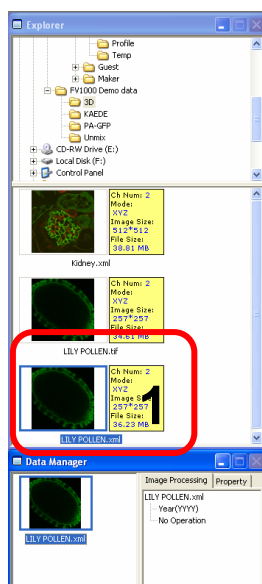
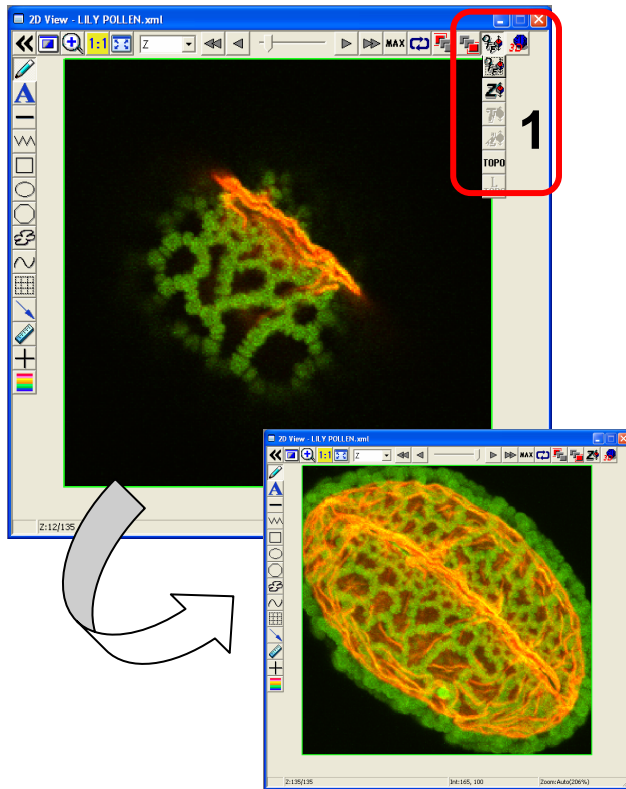


Image Analysis (Opening a File)

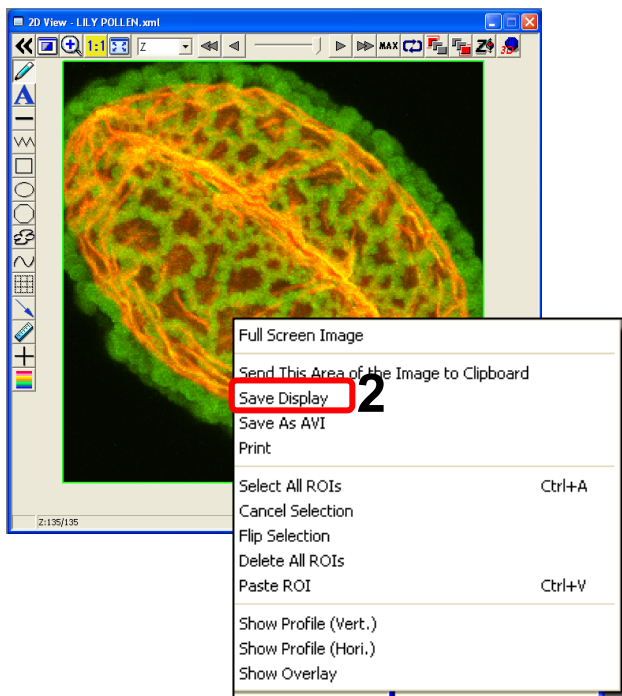


1. Double-click on a file to be opened from Explorer.

Image Analysis (Acquire a Projection Images)



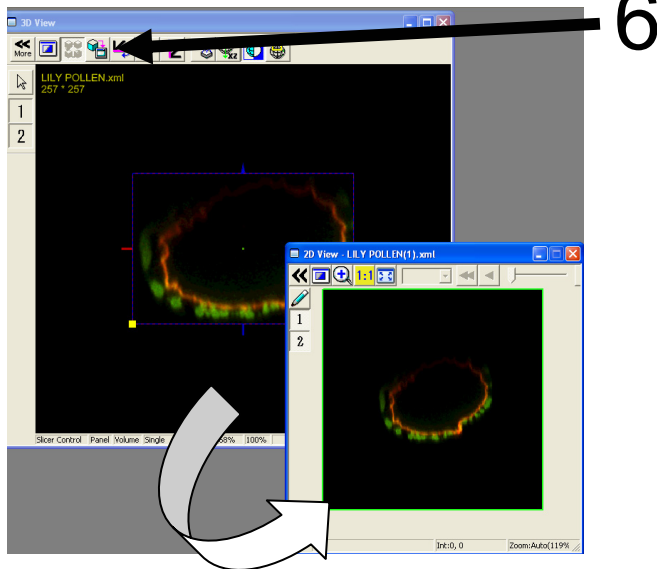
1. Click on the  button to select .



2. To save this image, right-click on the image, select Save Display and save the image with a new name.

Image Analysis

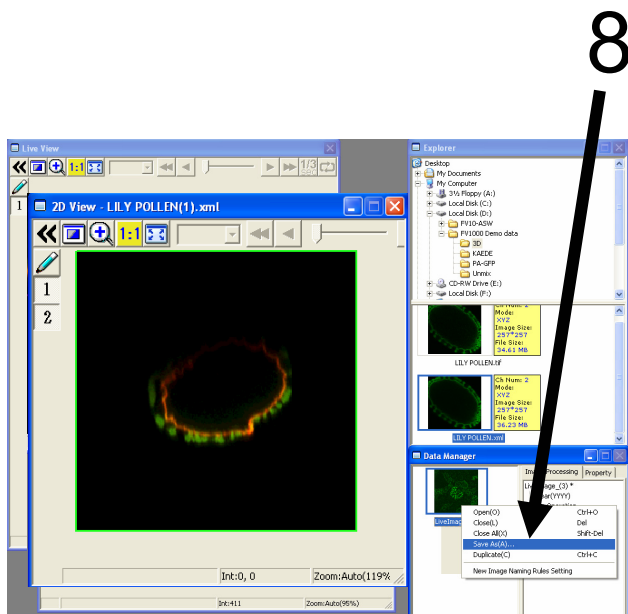
(Save a Z section Image as 2D file)



Save the image in step 3 or 5

6. Click on the  button.

7. A 2D View-(file name) image is created.



8. Saving the image:
Right-click on the Image Icon displayed on the Data Manager and select Save As to save the image. (Save as type "xml" is a file format specifically for the FV10-ASW software.)

■Memo■

File formats specifically for the FV10-ASW

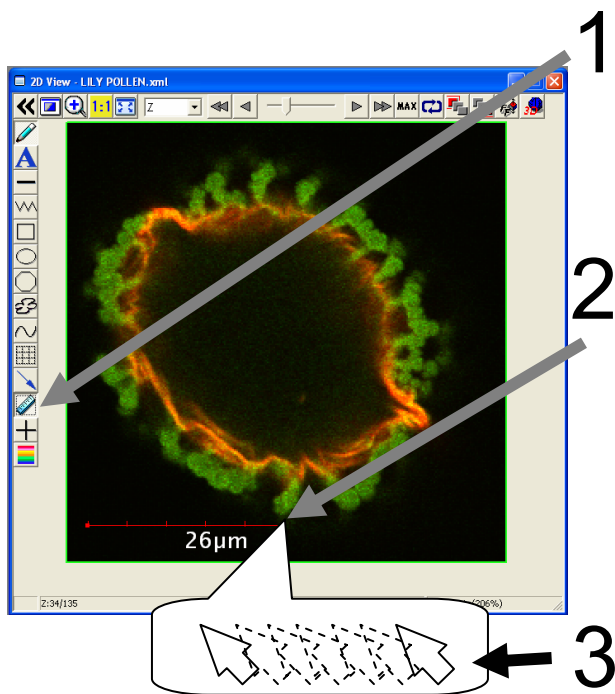
OIF format:

Creates "a folder that contains an image (16-bit TIFF)" and "an accessory file," which cannot be opened separately from each other.

OIB format:

Creates the OIF format files in a single file, which is convenient for migration and other operations.

Image Analysis (Inserting the Scale Bar)

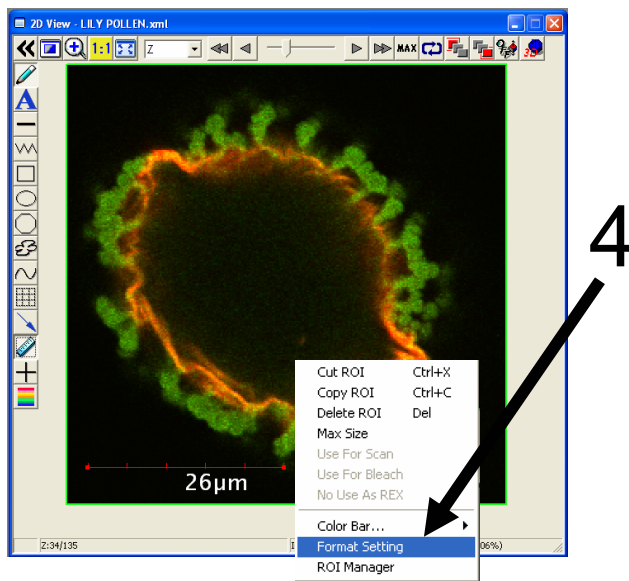


1. Click on the  button.

2. While left-clicking the image, drag and drop it at a certain point.

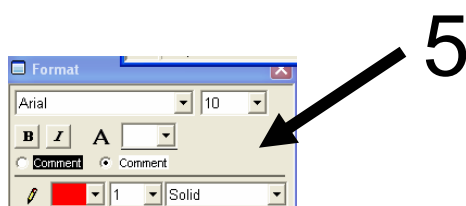
Change the size

3. While clicking the right or left handle, move the mouse from side to side.



Change the text size, color, style, etc.

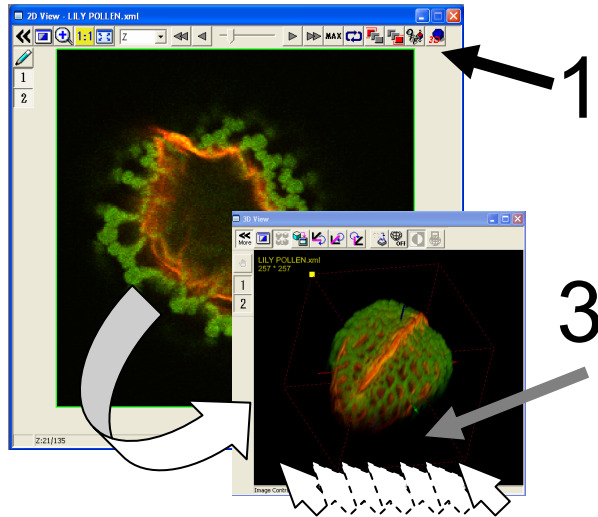
4. Select Scale Bar and then right-click on Scale Bar to select Format Setting.



5. Change the setting in this window as required.

Image Analysis

(Rotating a Three-dimensional Image)





1. Click on the  button for a 2D View-(file name) image.


2. A 3D view is created.

3. Drag the mouse on the image to observe it at a certain angle.

Simple animation

4. Press and hold the  button to rotate the image around the X-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Y-axis. Press it again to stop rotation.

Press and hold the  button to rotate the image around the Z-axis. Press it again to stop rotation.

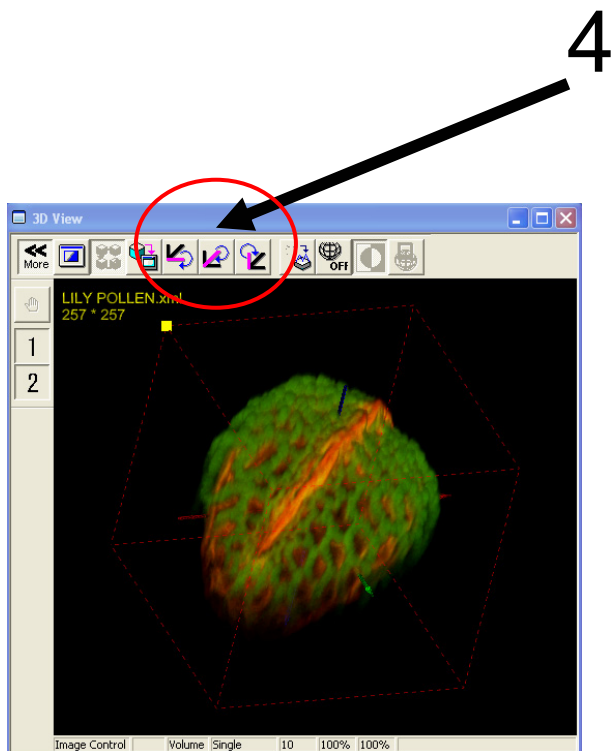
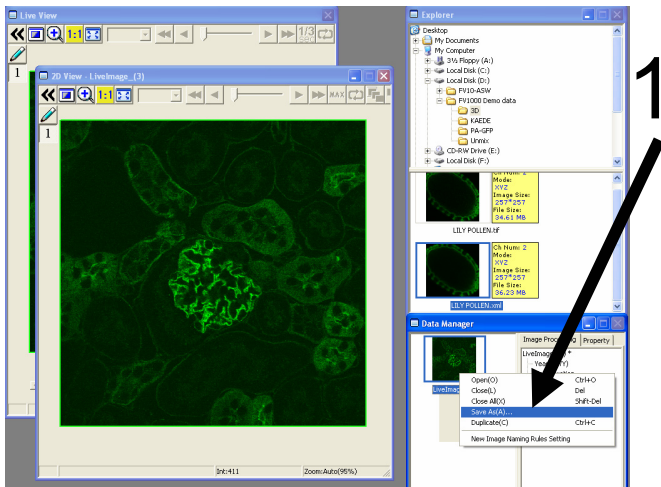


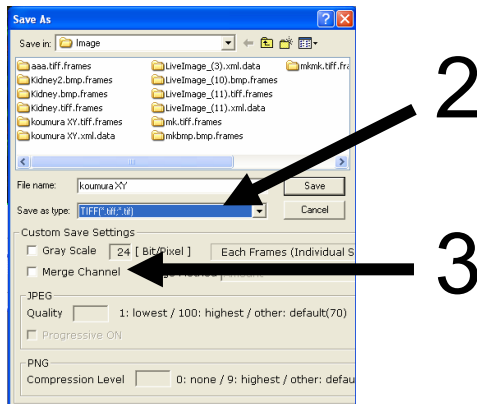
Image Analysis (Saving an Image)



Convert each channel of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to RGB Color.
4. Save the image.

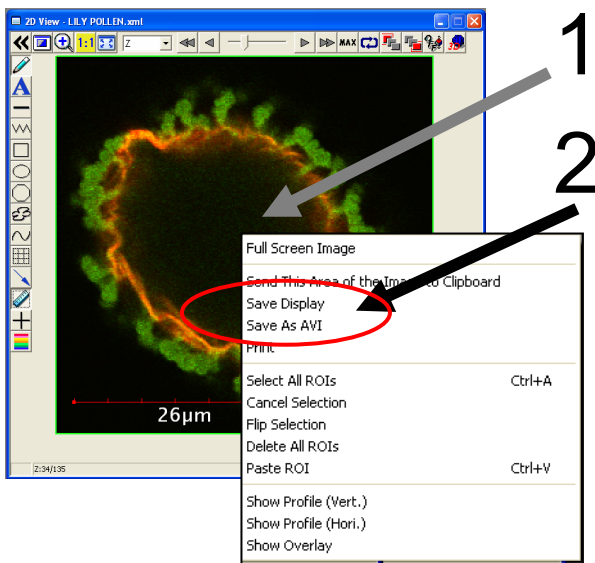
* BMP and JPEG formats are also selectable.



Convert a merge image of an XY or XYZ image into a TIFF format

1. Right-click on the Image Icon displayed on the Data Manager and select Export.
2. Set Save as type to TIFF.
3. Set Output Format to Merge Channel.
4. Save the image.

* BMP and JPEG formats are also selectable.



Convert an image with the scale bar inserted into a BMP format

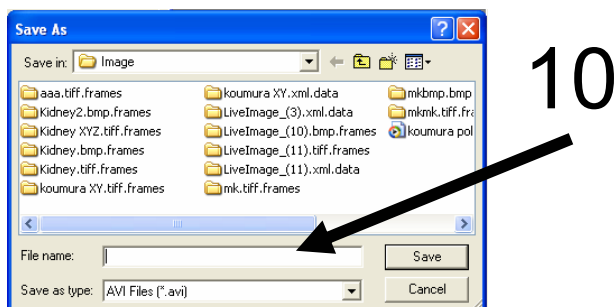
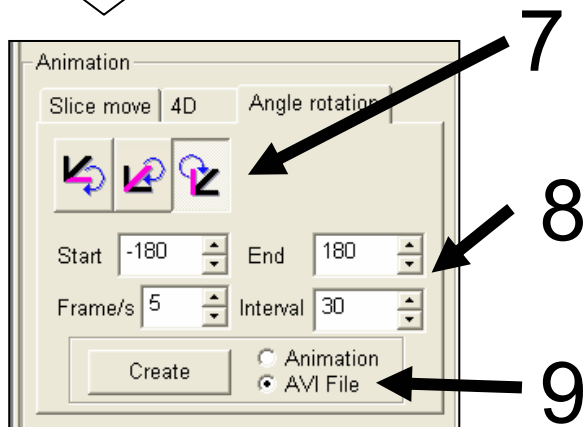
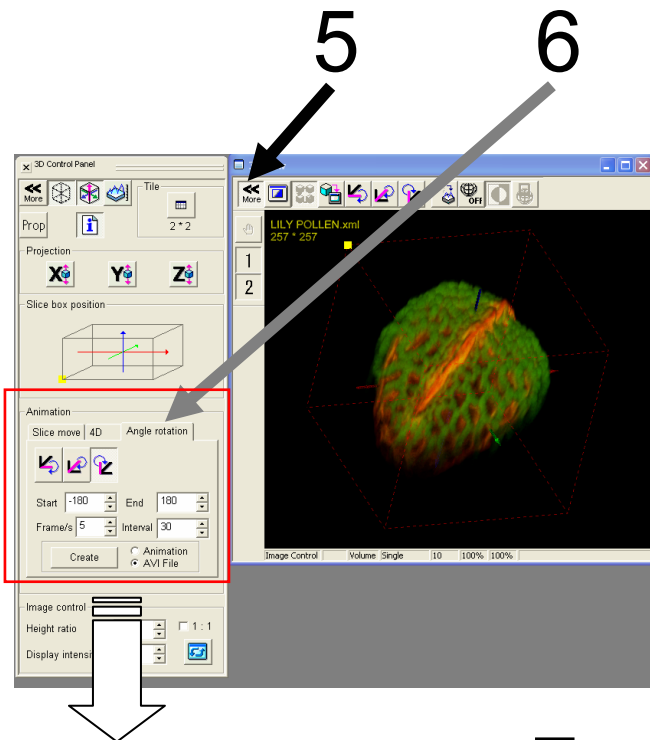
1. Right-click on the image.
2. Select Save Display and save the image with a new name.

Convert an animated image into an AVI format

1. Right-click on the image.
2. Select Save as AVI and save the image with a new name.

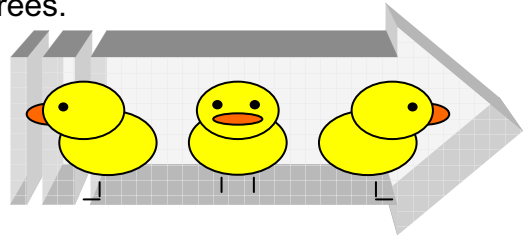
Image Analysis

(Rotating a Three-dimensional animation)



To save a rotation file as an animated image, create three-dimensional images according to the following procedure.

For example, try to rotate an image by 180 degrees.



5. Click on the  button.

6. Click on the Angle rotation tab.

7. Select the rotation axis.

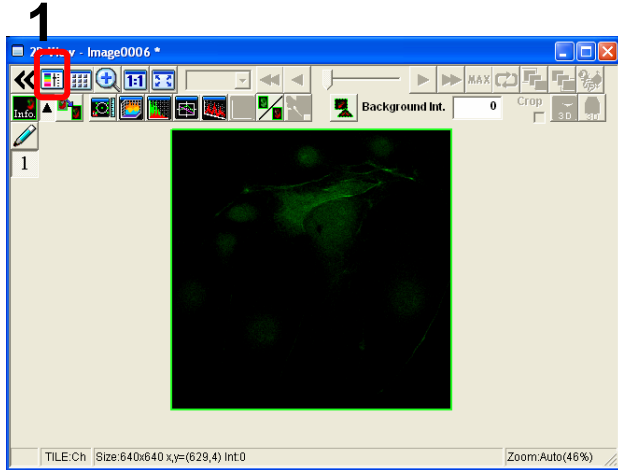
8. Enter the rotation angle.

Start = Angle to start rotation
End = Angle to stop rotation
Frame/s = Rotation speed
Interval = Degrees to be rotated at a time

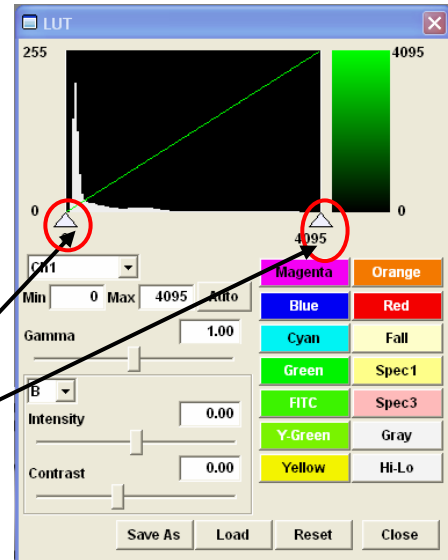
9. Select AVI File and click on Create.

10. Enter a file name and click on Save.

2D Image Analysis (Edit the image color and contrast)




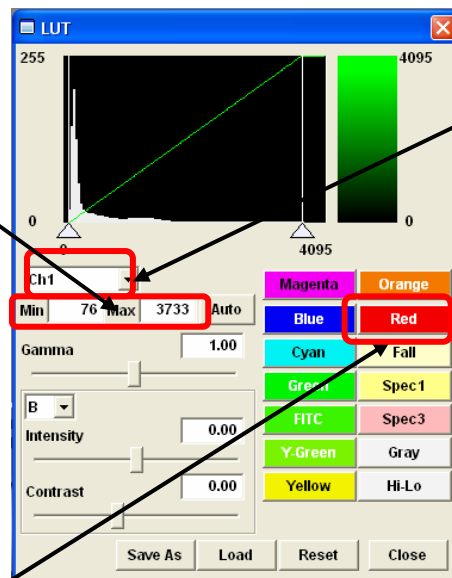
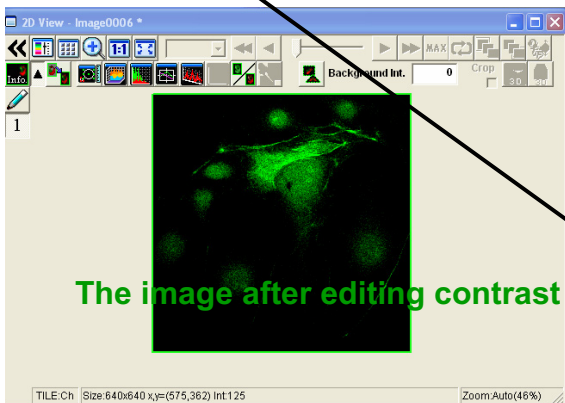
1. Click  "LUT" and then LUT table appears below,



3. Min and Max value are changed and contrast of image is edited.

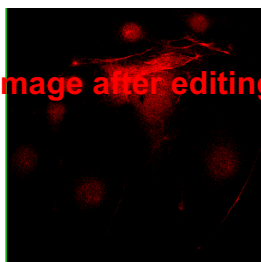
*According to get Min value up, be able to reduce noise of the image.

2. Edit contrast to drag  to left or right side, and another way to edit contrast is entering value on "Max" and "Min" (Max4095, Min0)



Edit each Ch

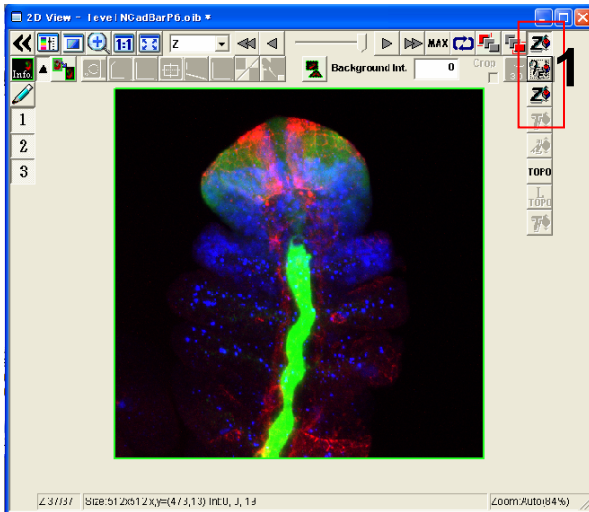
The image after editing color





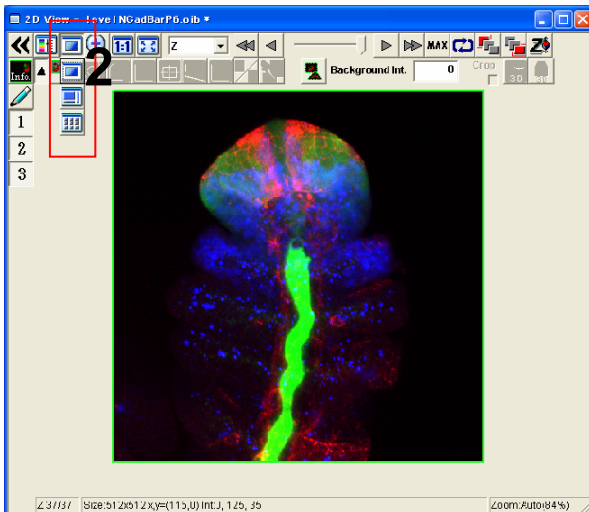
4. To click another color, be able to Edit a color. Above example: Change Green to Red to click



Red

2D Image Analysis (the image of Z section)

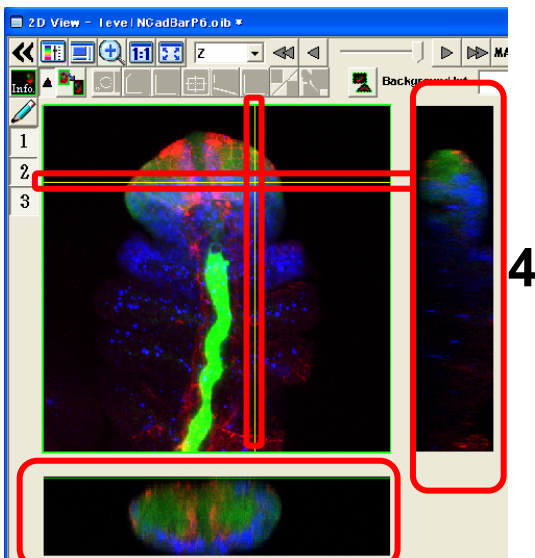


1. Click  and select  again, then Projection image is shown on 2D View after getting XYZ image.



2. Click  and select .

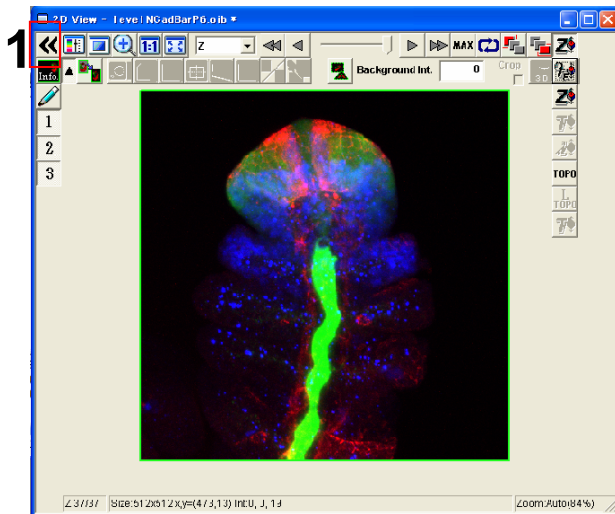
3. The images of Z section is shown on X axis and Y axis.
According to Move to left or right side on X axis and to move to ups and down on Y axis, be able to show image of Z section each position.




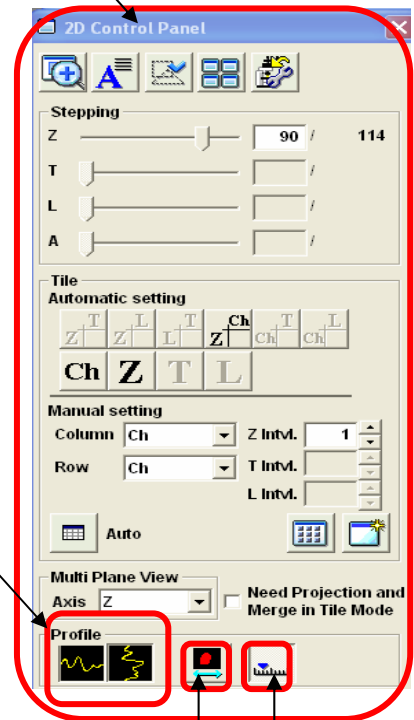
4. The image of Z section on Y axis.

5. The image of Z section on X axis.

2D Image Analysis (Intensity Profile of each Z sections)

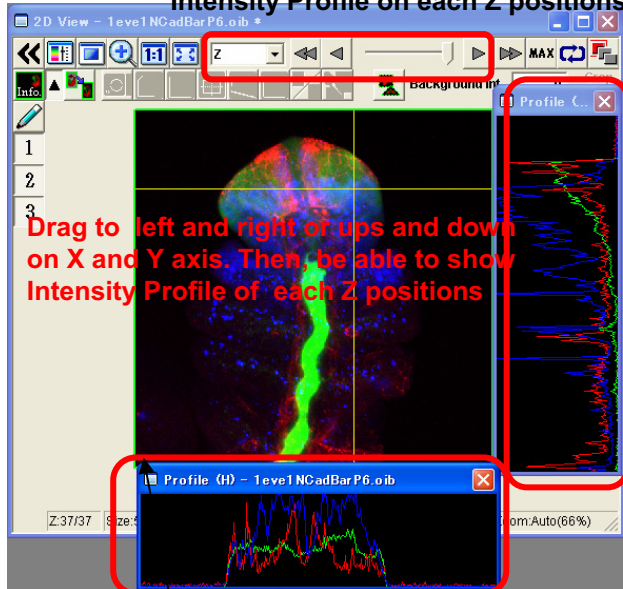


1. Click  and then 2D Control Panel is shown below,




2. Click  "Profile" and then Intensity Profile of each Z sections is shown on the X and Y axis.

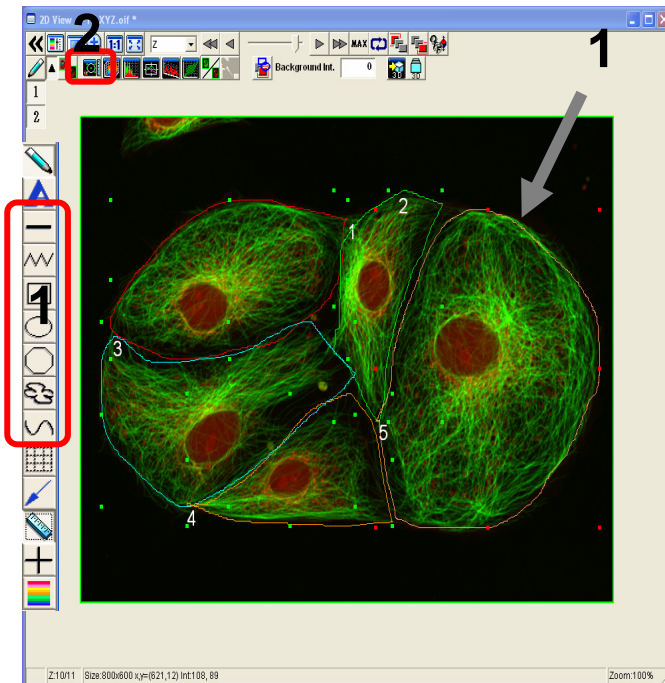
To move to Z position ,be able to show Intensity Profile on each Z positions.



3. Click  to show Scale on Intensity Profile

4. According to click , be able to show as equal scale of Profile window as 2Dimage.

2D Image Analysis (Measure)



1. Enclose interesting regions by ROI

Line on interesting positions by ROI

2. Click  "measure".

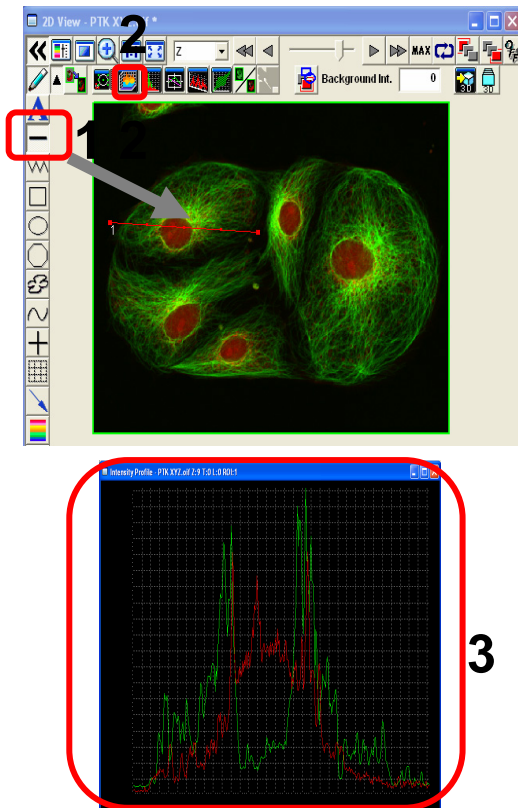
4. According to click "Measure All ROIs", then the information of all ROI is calculated on Region Measurement.

3. The information of ROI is calculated on Region Measurement.


5. The information of all ROIs

ROI	CenterX	CenterY	Area	Perimeter	Integration	Average	Max	Min	Range	StdDev	3StdDev	Integration	Average	Max	Min	Range	StdDev	3StdDev
	[um]	[um]	[um*2]	[um]	CHS1	CHS1	CHS1	CHS1	CHS1	CHS1	CHS1	CHS2	CHS2	CHS2	CHS2	CHS2	CHS2	CHS2
1	57.171	48.438	3129.625	241.490	5478264.000	1107.926	4095.000	95.000	4000.000	710.261	2130.783	2952481.000	658.076	3590.000	28.000	3562.000	522.518	1567.554
2	112.522	53.402	1470.188	194.764	9620457.000	1301.724	4095.000	97.000	3998.000	883.602	2650.807	7837013.000	758.280	3468.000	28.000	3440.000	561.877	1685.630
3	51.900	87.103	3274.688	273.215	2573667.000	1003.410	4095.000	94.000	4001.000	700.397	2101.192	9839166.000	569.504	3415.000	53.000	3362.000	443.623	1330.869
4	80.180	111.524	1732.438	211.246	4386227.000	879.766	3836.000	83.000	3753.000	657.656	1972.967	7880740.000	645.072	3380.000	25.000	3355.000	523.061	1569.183
5	150.780	79.732	6120.813	313.250	1878548.000	1244.509	4095.000	96.000	3999.000	725.103	2175.309	4771708.000	559.277	3227.000	41.000	3186.000	439.334	1318.002

2D Image Analysis (Line Intensity Profile on the 2D image)

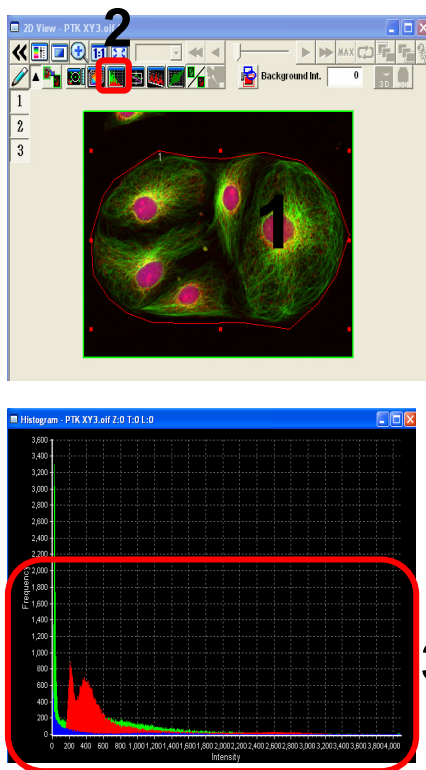


1. Line on the 2D image by ROI 

2. Click  “Intensity Profile”

3. “Intensity Profile” on the line is shown as intensity graph .

2D Image Analysis (Histogram)

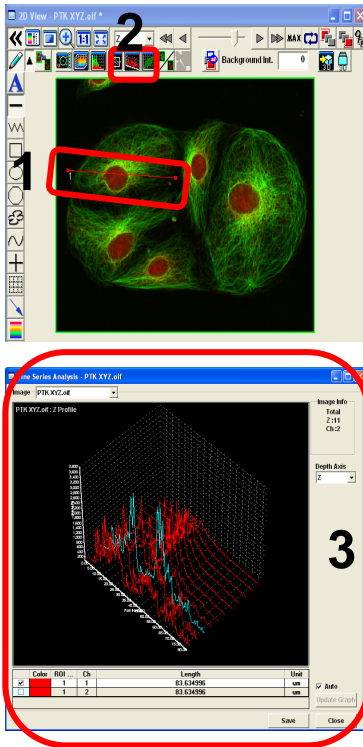


1. Enclose an interested area by ROI.


2. Click  “Histogram”

3. “Histogram” window is shown as a graph, frequency of intensity of each pixels is plotted on the area enclosed by ROI.

2D Image Analysis (Line Series Analysis)

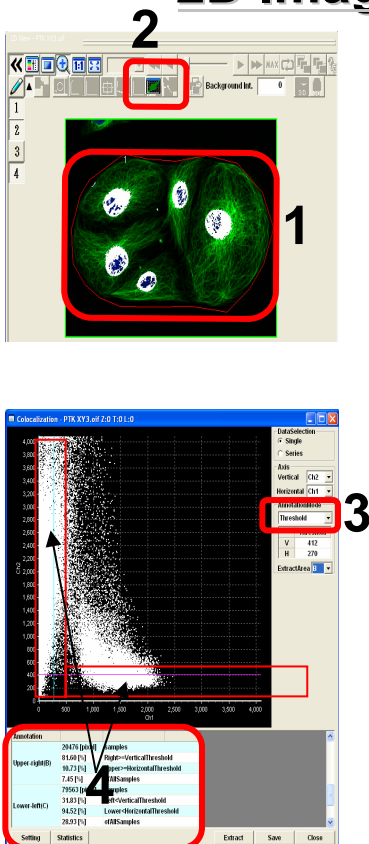


1. Line on the interesting region.

2. Click  “Line Series Analysis”


3. Intensity of Z position/ time on the line is shown as a graph .

2D Image Analysis (Co-localization)



1. Enclose an interesting region by ROI.

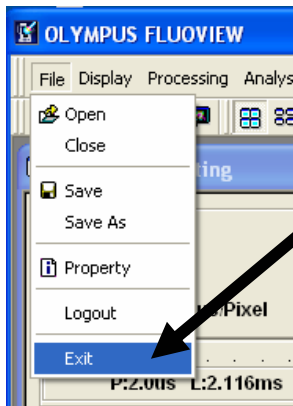
2. Click 

3. Select  **Threshold** from **Annotation Mode**.

4. According to move Thresholds of X,Y axis to right and left ,ups and down (**Enclose red color X,Y axis**), Co-localization result between 2ch is changed .

Information of Co-localization is listed under the scatter plot.

Closing the System



1. Exit the FV10-ASW software by selecting File/Exit.

2. Exit the Windows.

(1) Select Start/Shut Down.

(2) On the Shut Down Window, select Shut Down and click on OK.

3. Turn the laser OFF.

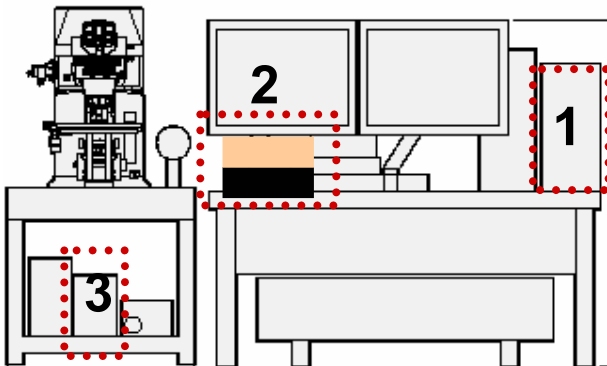
(Turn the key switch to the OFF position.)

3-1. LD559nm OFF

3-2. Multi Ar (458 nm, 488 nm, 514 nm) OFF

3-3. HeNe (G) (543 nm) OFF

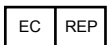
4. Turn the mercury burner power OFF.



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